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## Radical scavenging and antibacterial activity of *Arnebia benthamii* methanol extract

Showkat A Ganie<sup>1</sup>, Asima Jan<sup>2</sup>, Sabeera Muzaffar<sup>2</sup>, Bilal A Zargar<sup>3</sup>, Rabia Hamid<sup>1</sup>, M Afzal Zargar<sup>1\*</sup>

<sup>1</sup>Department of Biochemistry, University of Kashmir, Srinagar, India

<sup>2</sup>Department of Food Science and Technology, University of Kashmir, Srinagar, India

<sup>3</sup>Department of Pharmaceutical Sciences, University of Kashmir, Srinagar, India

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### ABSTRACT

**Objective:** To evaluate *in vitro* antioxidant and antibacterial activity of methanolic extract of *Arnebia benthamii* (*A. benthamii*) whole plant. **Methods:** Plasmid damage was analyzed by agarose gell electrophoresis. Calf thymus DNA was monitored by TBARS formation. DPPH, reducing power and lipid peroxidation was evaluated by using standard procedures. Antibacterial assay was monitored by disc diffusion method. **Results:** DPPH radical scavenging and hydroxyl radical scavenging potential of the plant revealed that the extract to be active radical scavenger. Reducing ( $Fe^{3+}$ - $Fe^{2+}$ ) power and lipid peroxidation inhibition efficiency (TBARS assay) of the extract was also evaluated and the extract showed promising activity in preventing lipid peroxidation and might prevent oxidative damages to biomolecules. The extract offered a significant protection against plasmid and calf thymus DNA damage induced by hydroxyl radicals. The extract was also evaluated on different bacterial strains and the maximum antibacterial activity was exhibited against *Escherichia coli* (*E. coli*) when compared with standard drug. **Conclusions:** These findings demonstrate that the methanol extract of *A. benthamii* has excellent anti-oxidant activities and could be considered as a potential source of lead molecules for pharmaceutical industries.

## 1. Introduction

Reactive oxygen species (ROS) are produced by cellular metabolism and by exogenous agents in the cells. These ROS may induce oxidative damage to various biomolecules in cells such as DNA, carbohydrates, proteins which in turn leads to cardiovascular and neurodegenerative diseases, inflammation and others<sup>[1,2]</sup>. Reactive oxygen species (ROS), which are generated from many redox processes, are major free radicals in the human body that are capable of inducing damage to biomolecules such as carbohydrates, proteins, lipids, and DNA<sup>[3]</sup>. Intracellular oxidative damage by ROS causes many chronic diseases, including neurodegenerative diseases and cardiovascular diseases<sup>[4,5]</sup>.

Living systems have specific pathways to overcome the adverse affects of various damages. However, sometimes

these repair mechanisms fail to keep pace with such deleterious effects<sup>[6]</sup>. An antioxidant is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate<sup>[7]</sup>. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infections and degenerative diseases. However, during recent years people have been more concerned about the safety of their food and the potential effect of synthetic additives on their health. The two most commonly used synthetic antioxidants; butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have begun to be restricted because of their toxicity and DNA damage induction<sup>[8,9]</sup>. Therefore, natural antioxidants from plant extracts have attracted increasing interests due to their safety. Natural antioxidants show strong defense against the damages of the cellular organelles caused by free radical induced oxidative stress<sup>[10]</sup>.

*Arnebia benthamii* (*A. benthamii*) is a monocarpic perennial and reaches reproductive maturity in 3–4 years.

\*Corresponding author: Dr. M Afzal Zargar, Department of Biochemistry, University of Kashmir, Srinagar, 190006, India.

Tel: +91-9419016466.

E-mail: zargarma@yahoo.co.in; zargarma@kashmiruniversity.ac.in

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The basal part of the root, leaves, and flowering stalk can be utilized for consumption and for trade. The roots yield a red pigment, shikonin and alkanin a lipophilic red pigment which is the main active constituent of this plant and are responsible for its colour and therapeutic efficacy. On folklore levels the plant is used for curing various diseases of tongue, throat, fever and cardiac disorders and has wound healing properties. The root has antihelminthic, antipyretic and antiseptic property. *A. benthamii* is used for imparting pleasing red colour to foodstuff, oils and fats. The flowering shoots are used in preparation of sherbets and jams.

The main objective of the present investigation was to evaluate the protective effects of methanolic extract of *A. benthamii* against free radical mediated damages under *in vitro* situations. *In vitro* assays were carried on DPPH radical scavenging activity, reducing power, •OH mediated DNA damage, lipid peroxidation and DNA nicking assay.

## 2. Materials and methods

### 2.1. Plant material collection and extraction

The *A. benthamii* was collected from higher altitudes of Gulmarg, and were identified by the courtesy of centre of Plant Taxonomy, Department of Botany, University of Kashmir. The whole plant material was shade dried under room temperature at  $(30 \pm 2)$  °C. The dried material was grinded into powder using mortar/pestle and sieved with a sieve of 0.3 mm aperture size. The powder obtained was extracted in methanol, by using Soxhlet extractor (60–80 °C). The methanol extract was then concentrated with the help of rotary evaporator under reduced pressure, and the solid extract was stored in refrigerator for further use.

### 2.2. DPPH radical scavenging activity: DPPH free-radical scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was measured by the method of Szabol[11]. The reaction mixture contained 10 mg% methanolic solution of DPPH and various concentrations of the test substances and were kept in dark for 50 min. Optical Density (OD) of the samples was measured at 517 nm against a blank. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.  $\alpha$ -tocopherol was taken as known free radical scavenger. Percentage inhibition was calculated by using the formula.

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where  $A_0$  was the absorbance of the control and  $A_1$  was absorbance in the presence of *A. benthamii* extract/known antioxidant.

### 2.3. Reducing power

The ability of the extracts to reduce  $Fe^{3+} - Fe^{2+}$  was accessed by the method of Yildirim[12]. 50  $\mu$ g, 100  $\mu$ g, 150  $\mu$ g, 200  $\mu$ g

and 250  $\mu$ g of the extracts were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6; 1.79%  $NaH_2PO_4$  and 1.89%  $Na_2HPO_4$ ) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 30 min. 2.5 mL of 10% trichloroacetic acid was later added and the tubes were centrifuged at 3 000 rpm for 10 min. 2.5 mL of the upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride added. Absorbance was measured at 700 nm. Increasing absorbance values of the reaction mixture indicated increasing reducing power of the extracts. BHT was taken as the known standard. The percentage reduction of the sample as compared with BHT was calculated by using formula:

$$\text{Reduction (\%)} = [1 - (1 - A_c/A_s)] \times 100$$

Where,  $A_c$  is absorbance of standard at maximum concentration tested and  $A_s$  is absorbance of sample.

### 2.4. Assessment of hydroxyl radical scavenging activity

Hydroxyl radical, generated from the  $Fe^{3+}$ -Ascorbate- $H_2O_2$  (Fenton reaction), was evaluated by degradation of deoxyribose that produced thiobarbituric acid reactive species (TBARS)[13]. The reaction mixture containing 25 mM deoxyribose, 10 mM Ferric chloride, 100 mM ascorbic acid, 2.8 mM  $H_2O_2$  in 10 mM  $KH_2PO_4$  (pH 7.4) and various concentrations of *A. benthamii* extract. The reaction mixture was incubated at 37 °C for 1 h. Then one mL of 1% thiobarbituric acid and 1 mL of 3% trichloroacetic acid were added and heated at 100 °C for 20 min. The TBARS was measured spectrophotometrically at 532 nm. The results were expressed as percentage inhibition of deoxyribose oxidation, as determined by the following formula.

$$\text{Percentage inhibition} = [(A-B)/A] \times 100$$

Where A was the malondialdehyde produced by Fenton reaction treated alone, and B was the malondialdehyde produced in the presence of *Arnebia benthamii* extract.

### 2.5. Lipid peroxidation (LPO)

LPO was induced and assayed in rat liver microsomes according to the method of Chang[14]. The liver of albino rats was homogenized with a homogenizer in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce. The homogenate was centrifuged at 100 000 g for 60 min at 4 °C, and the pellet was used for *in vitro* lipid peroxidation assay. An aliquot of microsomes (1 mL) was incubated with different concentrations of plant extract (final concentrations were 5, 10, 20, 50 and 100  $\mu$ g/mL, respectively) in the presence of 20 mM ferric nitrate, 100 mM ascorbic acid and 30%  $H_2O_2$  at 37 °C for 1 h. The reaction was ended by the addition of 1.0 mL of trichloroacetic acid (TCA; 28%, w/v) and 1.5 mL of TBA (1%, w/v), followed by heating at 100 °C for 15 min. The absorbance of the malondialdehyde (MDA) – TBA complex was measured at 532 nm.  $\alpha$ -tocopherol was used as positive controls.

The inhibition ratio (%) was calculated as % of inhibition = [(absorbance of control–absorbance of test sample)/absorbance of control] × 100% (17).

### 2.6. Antioxidant activity against oxidative damage to DNA

Hydroxyl radical generated by Fenton reaction were used to induce oxidative damage to DNA<sup>[15]</sup>. The reaction mixture (15  $\mu$  L) contained 25 mg of calf thymus DNA in 20.0 mM phosphate buffer saline (pH 7.4) and different concentrations of plant extract (10, 30, 50 and 80  $\mu$  g) were added and incubated with DNA for 15 min at room temperature. The oxidation was induced by treating DNA with 20 mM ferric nitrate and 100 mM ascorbic acid and incubated them for 1 h at 37 °C. The reaction was terminated by the addition of loading buffer bromophenol blue (0.25%) and glycerol (30%) and the mixture was subjected to gel electrophoresis in 0.7% agarose/TAE buffer run at 100 V. DNA was visualized and photographed by gel doc.

### 2.7. DNA nicking assay

DNA nicking assay was performed using supercoiled pBR322 DNA by the method of Leel<sup>[16]</sup>. A mixture of 5  $\mu$  L of plant extract of different concentrations (20–80 mg/mL) and plasmid DNA (0.5  $\mu$  g) was incubated for 10 min at room temperature followed by the addition of 10  $\mu$  L of Fenton's reagent (30 mM H<sub>2</sub>O<sub>2</sub>, 50 mM ascorbic acid and 20 mM FeCl<sub>3</sub>). The final volume of the mixture was made up to 20  $\mu$  L and incubated for 30 min at 37 °C. The DNA was analyzed on 1% agarose gel using ethidium bromide staining.

### 2.8. Antimicrobial activity

The disc diffusion method of Rahman and Rashid<sup>[17]</sup> was used to test antimicrobial activity of the methanol extract of *A. benthamii*. Solutions of known concentration ( $\mu$  g/mL) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette and the residual solvents were completely evaporated. Discs containing the test materials were placed on to nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of Kanamycin (30  $\mu$  g/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4 °C) for 24 h to allow maximum diffusion of the test materials and Kanamycin. The plates were then incubated at 37 °C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in

triplicate and the mean value was taken.

### 2.9. Statistical analysis

The values are expressed as mean  $\pm$  standard deviation (SD). The results were evaluated by using the SPSS (version 12.0) and Origen 6 softwares and each experiment was performed at least thrice.

## 3. Results

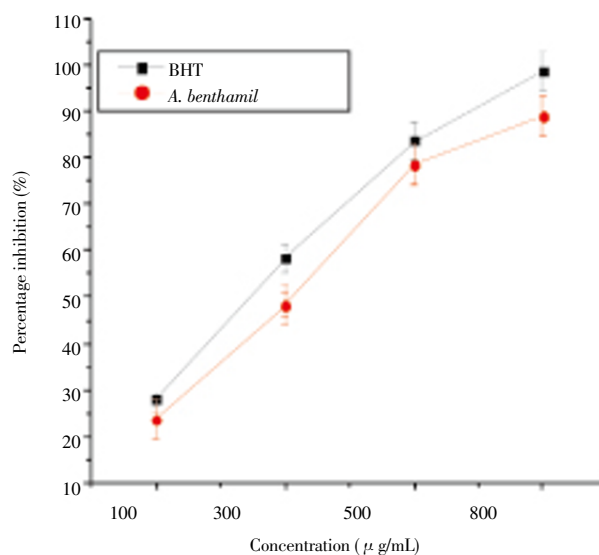
### 3.1. DPPH radical scavenging activity

To obtain information about the mechanisms of the antioxidative effects of the extract, we examined their radical scavenging effects by measuring changes in absorbance of DPPH radical at 517 nm. Both methanolic extract and BHT showed a concentration dependent scavenging of DPPH radicals. Methanolic extract was found to be more active radical scavenger than BHT.

Table 1 shows the results of the free radical (DPPH) scavenging activity in % inhibition. The results revealed that the methanol extract of *A. benthamii* exhibited the radical scavenging activity with (69.43  $\pm$  11.29) at the concentration of 700  $\mu$  g/mL. In comparison to BHT the known antioxidant which shows (85.10  $\pm$  1.34) at the same concentration.

### 3.2. Reducing power

The reducing power of methanolic extract of *A. benthamii* is summarized in Figure 1. The data shows that the reducing power of extract increased in a dose dependent manner. Methanolic extract displayed about 89% reducing activity as compared to the BHT which shows about 98% reducing power activity at the same concentration of 800  $\mu$  g/mL.



**Figure 1.** Represents the effect of *A. benthamii* methanol extract and known antioxidant BHT on reducing power method. The results represent mean  $\pm$  S.D of 3 separate experiments.

### 3.3. Hydroxyl radical scavenging activity

Antioxidant efficiency of the methanolic extract of *A. benthamii* was determined as their ability to scavenge the free radicals generated. The ability of the extract to quench hydroxyl radicals can be related to the prevention of lipid peroxidation, and it seems to be a good scavenger of active oxygen species, thus reducing the rate of chain reaction. Table 2 shows the percentage of hydroxyl scavenging activity (HRSA %) of *A. benthamii* extract. A dose dependent effect of the known antioxidant and our plant extract on hydroxyl radical scavenging was observed. At a concentration of 800  $\mu$ g/mL methanolic extract, 71% hydroxyl radical scavenging was observed.  $\alpha$ -tocopherol showed 90% hydroxyl radical scavenging activity at the same concentration. The experiment indicates that *A. benthamii* is an excellent hydroxyl radical scavenger and might play a role in neutralizing the lipid peroxidation.

### 3.4. Antioxidant activity of *A. benthamii* extracts on $Fe^{2+}$ /ascorbic acid model systems

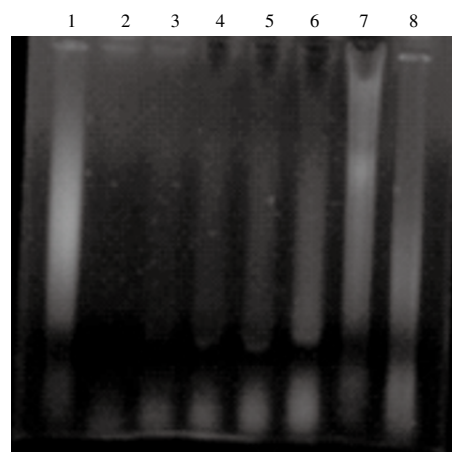
This model system contained  $Fe^{2+}$ /ascorbic acid as oxidizing agent to initiate lipid peroxidation in rat liver microsomes. MDA forms a pink chromogen with TBA that absorbs at 535 nm. *A. benthamii* extract dose dependently inhibited the MDA formation, and thus the lipid peroxidation in liver microsomes as shown in Table 3. At a concentration of 250  $\mu$ g/mL around 66.76% inhibitions in LPO was observed. In the presence of known antioxidant  $\alpha$ -tocopherol, LPO was inhibited by 87.25% at the same concentration.

### 3.5. Antioxidant activity against oxidative damage to DNA

The protective effect of *A. benthamii* methanol extract on calf thymus DNA is shown in the Figure 2. Hydroxyl radicals generated by Fenton reaction were found to induce DNA strand breaks in calf thymus DNA.  $H_2O_2$  alone did not cause DNA strand cleavage (lane 8). However  $H_2O_2$  in presence of ferric nitrate and ascorbic acid induce DNA strand breaks and helps the DNA molecule to run fast (lane 2). *A. benthamii* extract at 10–80  $\mu$ g offered complete protection to DNA damage induced by hydroxyl radicals in calf thymus DNA (lane 3–7). Thus, the hydroxyl radical quenching ability of polyphenolic compounds of *A. benthamii* could be responsible for the protection against oxidative damage to DNA.

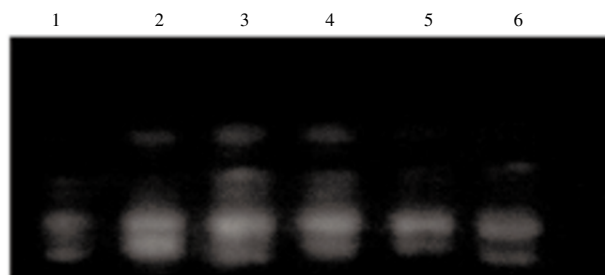
### 3.6. DNA nicking assay

Hydroxyl radicals generated by the Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms. In this assay when pBR322 plasmid DNA was exposed to Fenton reaction, it caused a damage in DNA molecule (lane 1). The concentration dependent (10–80  $\mu$ g/mL) free radical scavenging effect of methanolic extract of *A. benthamii* was studied (Figure 3) on plasmid DNA damage. The extract of higher concentration (80  $\mu$ g/mL) showed significant reduction in the formation of nicked DNA and increased native form of DNA (lane 6).



**Figure 2.** Protective effect of methanol extract of *A. benthamii* on oxidative damage to calf thymus DNA.

Lane 1: Native calf thymus DNA; Lane 2: DNA+ 20 mM Ferric Nitrate + 100 mM Ascorbic Acid + 30 mM  $H_2O_2$ ; Lane 3: DNA+ 20 mM Ferric Nitrate + 100 mM Ascorbic Acid + 30 mM  $H_2O_2$  + 10  $\mu$ g of methanol extract; Lane 4: DNA+ 20 mM Ferric Nitrate + 100 mM Ascorbic Acid + 30 mM  $H_2O_2$  + 20  $\mu$ g of methanol extract; Lane 5: DNA+ 20 mM Ferric Nitrate + 100 mM Ascorbic Acid + 30 mM  $H_2O_2$  + 30  $\mu$ g of methanol extract; Lane 6: DNA+ 20 mM Ferric Nitrate + 100 mM Ascorbic Acid + 30 mM  $H_2O_2$  + 50  $\mu$ g of methanol extract; Lane 7: DNA+ 20 mM Ferric Nitrate + 100 mM Ascorbic Acid + 30 mM  $H_2O_2$  + 80  $\mu$ g of methanol extract; Lane 8: DNA + 30 mM  $H_2O_2$  only.



**Figure 3.** Protective effect of methanol extract of *A. benthamii* on oxidative damage to pBR322.

Lane 1:  $H_2O_2$  + ferric nitrate+ ascorbic acid; Lane 2: Plasmid alone; Lane 3: Plasmid +  $H_2O_2$  + ferric nitrate+ ascorbic acid + 10  $\mu$ g/mL plant extract; Lane 4: Plasmid +  $H_2O_2$  + ferric nitrate+ ascorbic acid + 20  $\mu$ g/mL plant extract; Lane 5: Plasmid +  $H_2O_2$  + ferric nitrate+ ascorbic acid + 30  $\mu$ g/mL plant extract; Lane 6: Plasmid +  $H_2O_2$  + ferric nitrate+ ascorbic acid + 80  $\mu$ g/mL plant extract.

### 3.7. Anti bacterial activity

Four bacterial strains *E. coli*, *Klebsiella pneumonia* (*K. pneumonia*), *Staphylococcus aureus* (*S. aureus*) and *Shigella dysenteriae* (*S. dysenteriae*) were tested for their susceptibility to *A. benthamii* methanol extract. The standard antibacterial compound, Kanamycin is used at a concentration of 30  $\mu$ g/disc. The extract was tested at the concentration of 50  $\mu$ g/mL. Maximum zone of inhibition was observed with *E. coli* with inhibition zone diameter of 19 mm followed by *S. aureus* with inhibition zone diameter of 18 mm (Table 4).

The zone of inhibition was observed in the following descending order.

*E. coli* > *S. aureus* > *K. pneumonia* > *S. dysenteriae*

**Table 1**Shows the percentage inhibition of DPPH radicals by BHT, and different doses of methanolic extract of *A. benthamii*.

Concentration ( $\mu$ g/mL)	BHT	Methanolic extract of <i>A. benthamii</i>
100	14.13 $\pm$ 1.44	45.46 $\pm$ 2.66
200	21.04 $\pm$ 7.38	57.94 $\pm$ 1.04
300	22.25 $\pm$ 6.91	61.75 $\pm$ 1.09
400	33.66 $\pm$ 7.02	66.28 $\pm$ 1.50
500	43.67 $\pm$ 5.07	76.50 $\pm$ 0.76
600	46.60 $\pm$ 8.37	78.12 $\pm$ 1.86
700	69.43 $\pm$ 11.29	85.10 $\pm$ 1.34

The data were presented as means $\pm$ S.D of three independent experiments.**Table 2**

Shows the hydroxyl radical scavenging activity of plant extract and known antioxidant (Absorbance at 532 nm).

Concentration ( $\mu$ g/mL)	$\alpha$ – tocopherol	Methanolic extract of <i>A. benthamii</i> ( $\mu$ g/mL)
100	60.450 $\pm$ 0.039	27.003 $\pm$ 5.730
200	74.890 $\pm$ 0.035	36.390 $\pm$ 2.550
300	81.100 $\pm$ 0.015	44.100 $\pm$ 2.180
500	82.870 $\pm$ 0.069	52.670 $\pm$ 5.300
800	90.670 $\pm$ 0.007	71.290 $\pm$ 5.740

Absorbance of control 1.641 $\pm$ 0.170. The data were presented as means $\pm$ S.D of three independent experiments and evaluated by one-way ANOVA.**Table 3**Shows the effect of *A. benthamii* extract on MDA level in rat liver microsomes (nmoles /mg protein) (Absorbance at 535 nm).

Concentration ( $\mu$ g/mL)	$\alpha$ – tocopherol	Methanolic extract of <i>A. benthamii</i> ( $\mu$ g/mL)
50	50.170 $\pm$ 0.027	29.79 $\pm$ 12.85
100	57.900 $\pm$ 0.019	29.70 $\pm$ 11.76
150	63.130 $\pm$ 0.031	35.33 $\pm$ 10.10
200	78.040 $\pm$ 0.048	61.84 $\pm$ 6.70
250	87.250 $\pm$ 0.024	66.76 $\pm$ 4.93

The data were presented as means $\pm$ S.D of three independent experiments and evaluated by one-way ANOVA.**Table 4**Anti-bacterial activity of methanolic extract of *A. benthamii*.

S.No	Test bacteria	Volume of extract ( $\mu$ L)	Diameter of zone inhibition (mm)	Diameter of zone inhibition Kanamycin disc (mm)	Blank
1	<i>E. coli</i>	50	19.33 $\pm$ 1.52	18.00 $\pm$ 1.15	0
2	<i>S. aureus</i>	50	18.00 $\pm$ 2.08	19.00 $\pm$ 1.00	0
3	<i>K. pneumonia</i>	50	17.10 $\pm$ 1.00	18.00 $\pm$ 0.55	0
4	<i>S. dysenteriae</i>	50	17.00 $\pm$ 2.00	20.00 $\pm$ 1.09	0

The concentration of the Kanamycin used was 30  $\mu$  g/disc.

#### 4. Discussion

Antioxidant activity of *A. benthamii* extract has been revealed by free radical scavenging assays, DPPH radical scavenging assays, Reducing power, hydroxyl radical scavenging activity, lipid peroxidation assays and hydroxyl radical-induced DNA and plasmid damage. In addition, *A. benthamii* extract has high phenolic contents. This study shows that *A. benthamii* extract clearly has antioxidant effects. DPPH is a stable radical that has been widely utilized to appraise the antioxidant activity of various natural products<sup>[18]</sup>. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, e.g., a free radical-scavenging antioxidant, the absorption strength is decreased and results in decolorization (yellow

colour) with respect to the number of electrons captured<sup>[19]</sup>. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. In this study, DPPH scavenging activity has been found in *A. benthamii* extract. The scavenging effects of extract increased with their concentrations to similar extents. The percentage inhibitions of concentration 100–700  $\mu$  g/mL are about 14%, 21%, 22%, 33%, 43%, 46% and 69.43% respectively as shown in Table 1. The standard BHT presented a scavenging effect of 85% at the concentration of 700  $\mu$  g/mL. Similar results were reported by Ganie *et al*<sup>[20]</sup> while investigating the DPPH radical scavenging activity of *Podophyllum hexandrum*.

The ability of the extract and BHT to reduce the ferric–ferricyanide complex to the ferrous–ferricyanide complex of Prussian blue was determined by recording the 700 nm absorbance after incubation. Reductants supply electrons to the reduced species and, in antioxidant substances,

reductants cause the reduction of the  $\text{Fe}^{3+}$ /ferricyanide complex to the ferrous form, causing the test solution to change color from yellow to green or blue, based on the substance's reducing ability<sup>[21]</sup>. In this study, there was a steady increase in the reductive potential of the extract and BHT with increased concentration, but the extract showed lower activities than the synthetic antioxidant. The extract may have acted as a scavenger by neutralizing free radicals through donating electrons and rendering the radicals stable, thus also quenching the free radical chain<sup>[22]</sup>. Similar results were reported by Ganie *et al.*<sup>[23]</sup> for 70% ethanol extract of *Podophyllum hexandrum* with high relationship between phenolic compounds and reducing power of the extract.

Hydroxyl radical is an extremely reactive species formed in biological systems. It is capable of damaging almost every molecule found in living cells<sup>[24]</sup>. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. *In vitro*,  $(\text{OH}^\cdot)$  were generated by a mixture of  $\text{Fe}^{3+}$ -EDTA,  $\text{H}_2\text{O}_2$  and ascorbic acid and were assessed by monitoring the degraded fragments of deoxyribose, through malonaldehyde (MDA) formation. If any drug scavenges the hydroxyl radical, they may either scavenge the radical or may chelate the  $\text{Fe}^{2+}$  ion making them unavailable for the Fenton's reaction. Here the methanol extract of *A. benthamii* was tested first time in our lab, where we found a dose dependent hydroxyl radical scavenging activity. The methanol extract of *A. benthamii* was found to exhibit the scavenging activity with 71.29% at 800  $\mu\text{g/mL}$  concentration. The scavenging effect was comparable to that of the standard Vitamin E with 90.67% at 800  $\mu\text{g/mL}$  concentration. Similar studies conducted on *Cassia fistula* showed that the extract provided 30% protection against deoxyribose oxidative damage at a concentration of 125  $\text{mg/mL}$ <sup>[25]</sup>.

Peroxidation is important in food deterioration and in the oxidative modification of biological molecules particularly lipids. Inhibition of lipid peroxidation by any external agent is often used to evaluate its antioxidant capacity. In the present study the inhibition of lipid peroxidation induced by  $\text{H}_2\text{O}_2$ /ascorbic acid/ferric nitrate by the *A. benthamii* methanol extract was carried out in rat liver microsomes. Several plant extract/constituents have been found to exert their antioxidant activity by chelating the Fenton generated free radicals and also inhibit LPO<sup>[26–27]</sup>. *A. benthamii* extract exhibited dose dependent inhibitory effects on the *in vitro* rat liver microsomal lipid peroxidation. The extract afforded 66.76% protection at the concentration of 250  $\mu\text{g/mL}$  and the known antioxidant  $\alpha$ -tocopherol exhibited 87.25% at the same concentrations. Our results are in good agreement with the reports on medicinal plants as an important supplement for reducing hyperglycemia due to inhibition of lipid peroxidation process, owing to several types of phenolic compounds and antioxidant activities<sup>[28]</sup>.

The cellular damage resulting from hydroxyl radicals is strongest among free radicals. Hydroxyl radicals can be generated by biochemical reaction. Superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently produce extremely reactive hydroxyl radicals in the presence of transition metal ions such as iron and copper or by UV photolysis. DNA is susceptible to oxidative damage and hydroxyl radicals oxidize guanosine or thymine to 8-hydroxyl-2-deoxyguanosine and thymine glycol which change DNA and lead to mutagenesis and carcinogenesis<sup>[29]</sup>. In this study, hydroxyl radicals generated

by Fenton reaction were found to induce DNA strand breaks in calf thymus DNA and uncoiling of supercoiled plasmid DNA. *A. benthamii* methanol extract at 10–80  $\mu\text{g}$  offered complete protection to DNA damage induced by hydroxyl radicals in calf thymus DNA and also reduced uncoiling or open circular form in pBR322 DNA. Thus, the hydroxyl radical quenching ability of polyphenolic compounds of *A. benthamii* extract could be responsible for the protection against oxidative damage to DNA<sup>[30–35]</sup>.

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial anti-microbial drugs commonly used in the treatment of infectious diseases<sup>[36]</sup>. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions<sup>[37]</sup>. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants<sup>[38]</sup> as well as develop methods to decrease the concentrations of the drugs used. Several screening studies have been carried out in different parts of the world. The beneficial medicinal effects of plant materials typically results from the combination of secondary products present in the plants. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, coumarins and phenolic compounds. From the data it is evident that methanolic extract of *A. benthamii* is active against all the four tested strains of bacteria, but more active against gram negative bacteria such as *E. coli*. Similar results were reported by Emin Baby<sup>[38]</sup> while investigating the antibacterial activity of methanolic extract of *Passiflora foetida* Linn.

From the results obtained in the present study, it is concluded that methanolic extract of *A. benthamii* exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. The antibacterial activity in methanolic extract of *A. benthamii* by disc diffusion method showed good antibacterial activity against above discussed bacteria. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract and to isolate the main components responsible for these antibacterial activities. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

### Conflict of interest statements

The authors suggest that they have no conflict of interest.

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