Antioxidant and Anti-inflammatory Activities of *Aralia cachemirica* Decne.: A Comparative Study

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Abstract

**Background:** *Aralia cachemirica* Decne. is an important medicinal plant species of Kashmir Himalaya. Regardless of having enormous medicinal importance little information exists on the biological activities of the species. For this reason, present work was carried out for providing comprehensive details on the antioxidant and anti-inflammatory activities of different parts of *A. cachemirica*.

**Methods:** In this connection, different parts (leaves, stem and root) of the plant were examined following standard methodologies. Antioxidant potential of the plant was determined by three different standard methodologies viz. DPPH radical scavenging activity, reducing power activity and metal chelating activity. For anti-inflammatory activities two different standard methods viz. human RBC membrane stabilization method and protein denaturation method were followed.

**Results:** The extracts exhibited concentration dependent antioxidant and anti-inflammatory activities but were weaker than the standards. Also, the highest activities were recorded in the root and least in the leaves. Also, the highest activities were recorded in the leaves and least in the stem.

**Conclusion:** This is the first study providing information regarding antioxidant and anti-inflammatory potential of different parts of the species. The antioxidant activity of the extract could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated, oxidative stress related degenerative diseases. Plant derived anti-inflammatory agents are competent cure for inflammation caused by a range of agents, which shows the way to their soaring requirement. Exploitation of these agents requires to be regulated in order, to be sustainable and keep up with demands.

**Background**

Antioxidants are the chemical compounds that may guard cells from the harm caused by unstable molecules known as free radicals. These interact with and stabilize free radicals and thus may avoid some of the damage free radicals might otherwise cause. The damage caused by free radicals may lead to cancer. Lycopene, beta-carotene, vitamins C, E, A etc. are some of the examples of antioxidants [1]. Reactive oxygen species (ROS) in the human body are...
implicated in varied physicochemical processes [2]. ROS are responsible mainly for pathogenesis of different diseases such as neurodegenerative disorders, cancer, artherosclerosis, cardiovascular diseases, cataracts and inflammation [3].

Inflammation is a host defense machinery of the body and it’s a vital immune response that allows body survival during infection or injury and retains tissue homeostasis in toxic conditions. According to the modern notion, inflammation is a healthy process resulting from various disturbances or diseases. Inflammation is a common reaction to any noxious stimulus that threatens the host and may differ from localized response to a generalized one [4]. In other words “Inflammation is the chief and complex reaction of the body against infection upon tissue injury.” Today the role of inflammation as a restorative process, healing, as well as its aggressive role, is more extensively recognized. However, in some conditions inflammation appears to be no resolution and a chronic state of inflammation develops that may be fatal for an individual. Such conditions include the inflammatory disorders like osteoarthritis, rheumatoid arthritis, retinitis, inflammatory bowel diseases, psoriasis, multiple sclerosis and atherosclerosis. Different kinds of safe and effective anti-inflammatory agents are available to overcome this problem, such as aspirin and other nonsteroidal anti-inflammatory agents, including various drugs which are under development. As such, the agents which are helpful to reduce the inflammatory response are known as anti-inflammatory agents [5]. The symptoms of inflammation are characterized by pain, heat, redness, swelling and loss of function. These symptoms result from the dilation of the blood vessels which leads to an increased blood supply and increased intracellular spaces hence, resulting in the movement of leukocytes, protein and fluids into the inflamed regions [6,7].

*Aralia cachemirica* Decne. commonly known as “Kashmir Spikenard” and locally known as “Khoree” belongs to the family Araliaceae. It is a shrubby herb, 1 to 3 m tall and is found distributed in temperate Himalayas from Kashmir to Sikkim at 2100 to 4000 m altitude [8,9]. The following phytocryptitations have already been isolated from the plant; Octadec-6-enoic acid, 8-primara-14, 15-diene-19-oic acid, Aralosides A&B [10,11]. Anti-inflammatory activity also has been reported in the roots of the plant [11]. Bhat *et al.*, [11] reported hypoglycemic activity from the roots of *Aralia cachemirica*. Furthermore, isolation of continentalic acid from *A. cachemirica* and its immunomodulatory activity has already been reported [12].

Although having great medicinal importance little information is available on the biological activities of *A. cachemirica*. Hence, present work can only be an attempt for providing comprehensive report on the comparative estimation of antioxidant and anti-inflammatory potential of *A. cachemirica*. In this connection, different parts (leaves, stem and root) of the plant were examined following standard methodologies.

**Methods**

**Preparation of plant extract**

Healthy and disease free plants of *A. cachemirica* were collected from Ferozpur Nallah area of Jammu and Kashmir. The collected specimens were identified and deposited in Kashmir University Herbarium (KASH) under voucher number 2689-KASH. The plant collections were made prudently throughout the course of the present study.
The plant materials were fragmented into different parts (root, stem and leaves) and dried under shade at room temperature for 15-20 days. After shade drying, the plant materials were pulverized to coarse powder using grinder. Soxhlet extraction was carried out using hydroalcoholic solvent (70% ethanol) and extract obtained. The extracts were filtered through Whatmann filter paper No.1. The pellet was discarded and the supernatant was collected and concentrated under thermostat water bath at required temperature. Finally the extracts were dried, labeled and stored at 4˚C in storage vials for further experimental uses.

Antioxidant activity assays

DPPH radical scavenging activity

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method [13] with suitable modifications. The stock solution of extracts were prepared in methanol to achieve the concentration of 1mg/ml. Dilutions were made to obtain concentrations of 100-600µg/ml. Diluted solutions (1ml each) were mixed with 1ml of methanol solution of DPPH in concentration of 1mg/ml. After 30min incubation in darkness at room temperature, the absorbance was recorded at 517nm. Control sample contains all the reagents except the extract.

\[ \text{DPPH free radical inhibition (\%) } = \left( \frac{A_c - A_s}{A_c} \right) \times 100 \]

Where, Ac is the absorbance of control and As is the absorbance of sample.

L-Ascorbic acid was used as standard antioxidant and served as positive control.

Reducing power method

Total reducing power of the plant extracts was determined by Oyaizu, [14] method. Extracts of different concentrations were taken (100, 200, 300, 400, 500 and 600µg/mL) and to these plant extracts 1% potassium ferricyanide (2.5ml) were added. Also, phosphate buffer (2.5ml) (0.2M, pH 6.6) was added and the mixture was incubated at 50°C for 20min 10% TCA (2.5ml) was added to the reaction mixture which was centrifuged at 1000rpm for 10min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml), FeCl3 (0.5ml, 0.1%) and the absorbance was measure at 700 nm. Ascorbic acid (10-100µg/ml) was used as positive control. Percentage inhibition was calculated for the determining reducing power.

\[ \% \text{ Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Metal chelating activity

The chelation of ferrous ions by extracts was estimated by method of Dinis et al. [15]. 50µl of 2mM FeCl₂ was added to 1ml of different concentrations of the extracts (100, 200, 300 400, 500 and 600µg/ml). The reaction was initiated by the addition of 0.2ml of 5mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10min. The absorbance of the solution was thereafter measured at 562nm. The percentage inhibition of ferrozine-Fe²⁺ complex formation was calculated as
\[
\left(\frac{A_0 - A_s}{A_0}\right) \times 100
\]

Where, \(A_0\) is the absorbance of control and \(A_s\) is the absorbance of sample.

Citric acid was used as standard and served as positive control.

**Anti-inflammatory activity**

**Human RBC membrane stabilization method Sadique et al., [16]**

The human red blood cell membrane stabilization method was used for the study. The fresh blood (10ml) is collected from a healthy human volunteer who has not taken any NSAIDS for 2 weeks prior to the experiment and transferred to the heparinized centrifuge tubes. The tubes were centrifuged at 3000rpm for 10min and were washed thrice with equal volume of normal saline and 10% v/v suspension was prepared. Various concentrations of extracts were prepared (100, 200, 300, 400, 500 and 600µg/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension were added. It is incubated at 37°C for 30min and centrifuged again at 3000rpm for 20 min. The haemoglobin content of the supernatant solution is estimated spectrophotometrically at 560nm. Indomethacin (100µg/ml) was used as a reference standard. The percentage of HRBC membrane stabilization was calculated by using formula:

\[
\frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs control}} \times 100
\]

Where, Abs = Absorbance

**Protein denaturation method Sakat et al., [17]**

Protein denaturation was used as an *in-vitro* method to study the anti-inflammatory activity. The reaction mixture (5ml) consisted of 0.2 egg albumin (from fresh hen’s egg), 2.8ml phosphate buffered saline (PBS) (pH 6.4) and 2ml varying concentrations of test extracts (100, 200, 300, 400, 500 and 600 µg /ml). The sample extracts were incubated at 37 °C for 15min and then heated at 70°C for 5min. After cooling the samples the absorbance was measured at 660nm using vehicle as blank. Indomethacin (100µg/ml) was used as a reference standard. The Percentage inhibition of protein denaturation was calculated by using the formula:

\[
\text{Percentage inhibition} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs control}} \times 100
\]

Where, Abs = Absorbance

**Results**

Soxhlet extraction of the Aharbal population was performed using hydroalcoholic solvent (70% ethanol). The antioxidant capacity of extracts of the plant *A. cachemirica* various parts was assessed by employing three different methods. The results are compared with the standard antioxidants. The extracts exhibited concentration dependent antioxidant activity but were weaker than the standards.
DPPH radical scavenging activity in crude extract of *A. cachemirica*

The DPPH radical scavenging activity in various parts of *A. cachemirica* is presented in the table 1 and fig. 1. The DPPH radical scavenging activity was highest in the leaves followed by the root and least in the stem. Also, maximum antioxidant activity is recorded at 600 µg/ml concentration in all the plant parts.

**Table 1: Comparison of DPPH radical scavenging activity in different parts of *Aralia cachemirica***

| Conc. (µg/ml) | L-ascorbic acid | Plant parts % inhibition |
|---------------|-----------------|-------------------------|
|               |                 | Leaf | Stem | Root  |
| 100           | 73.67±0.472*    | 53.43±1.11 | 43.18±1.09 | 50.38±1.05 |
| 200           | 77.89±0.148     | 57.75±0.90 | 46.55±1.00 | 53.81±0.96 |
| 300           | 82.76±0.343     | 63.59±0.75 | 51.43±1.08 | 58.27±1.05 |
| 400           | 86.34±0.260     | 70.18±1.01 | 54.72±1.02 | 64.75±0.97 |
| 500           | 91.45±0.153     | 75.14±0.90 | 58.58±1.01 | 68.38±1.04 |
| 600           | 96.87±0.087     | 79.44±0.79 | 60.67±1.02 | 71.63±0.97 |

*Mean (triplicates) ±SD

Fig. 1: Graph showing comparative percent inhibition of DPPH radical scavenging activity in different parts of *Aralia cachemirica*.

Reducing power activity in crude extract of *A. cachemirica*
The reducing power activity in various parts of A. cachemirica is presented in the table 2 and fig. 2. Also, the reducing power activity is highest in the leaves followed by the root and least in the stem. Furthermore, maximum antioxidant activity is recorded at higher concentrations i.e., 600 µg/ml in all the plant parts.

### Table 2: Comparison of % inhibition of reducing power activity in various parts of Aralia cachemirica

| Conc. (µg/ml) | L-ascorbic acid | Plant parts % inhibition |
|---------------|-----------------|--------------------------|
|               | Leaf            | Stem                     | Root                      |
| 100           | 0.399±0.002     | 0.361±0.001              | 0.245±0.001               |
| 200           | 0.472±0.003     | 0.435±0.001              | 0.264±0.001               |
| 300           | 0.556±0.003     | 0.475±0.001              | 0.271±0.002               |
| 400           | 0.671±0.002     | 0.485±0.001              | 0.291±0.001               |
| 500           | 0.742±0.002     | 0.508±0.001              | 0.305±0.001               |
| 600           | 0.875±0.002     | 0.539±0.001              | 0.306±0.001               |

*Mean (triplicates) ±SD

![Fig. 2: Graph showing comparative percent inhibition of reducing power activity in different parts of Aralia cachemirica](image)

Metal chelating activity in crude extract of A. cachemirica

The metal chelating activity in various parts of A. cachemirica is presented in the table 3 and fig. 3. The results reveal that the percentage of metal chelating activity is highest in the leaves (70.02±1.04 µg/ml) followed by the root (67.17±1.09 µg/ml), and least in the stem (59.19±1.05 µg/ml). Also, maximum antioxidant potential is recorded at higher concentrations i.e., 600 µg/ml in all the plant parts.

### Table 3: Comparison of % inhibition of metal chelating activity in various parts of Aralia cachemirica

| Conc. (µg/ml) | Citric acid | Plant parts % inhibition |
|---------------|-------------|--------------------------|
|               |             |                          |

*Fig. 3: Graph showing comparative percent inhibition of metal chelating activity in various parts of Aralia cachemirica*
Fig. 3: Graph showing comparative percent inhibition of metal chelating activity in different parts of *Aralia cachemirica*

**In vitro** anti-inflammatory activity

The *in vitro* anti-inflammatory activity of various plant extracts of *A. cachemirica* is determined by employing two different methods. The results are compared with the standards. The results depicted concentration dependent anti-inflammatory activity in various plant extracts but were weaker than the standards.

**Protein denaturation method**

The result of % inhibition of protein denaturation in various parts of *A. cachemirica* is presented in the table 4 and fig. 4. It is evident from the table that the leaves show highest % inhibition of protein denaturation followed by the root and then the stem. Also, from the table it is clear that as the concentration of the extracts increases the protein denaturation % inhibition also shows an increase.

**Table 4: Comparison of % inhibition of protein denaturation in different parts of *Aralia cachemirica***
| Conc. (µg/ml) | Indomethacin | Plant parts % inhibition |
|--------------|--------------|-------------------------|
|              | Leaf | Stem | Root  |
| 100          | 65.32±0.561* | 54.03±1.15 | 48.21±1.15 | 51.29±1.15 |
| 200          | 68.87±0.473 | 60.11±1.11 | 51.40±0.91 | 55.36±0.83 |
| 300          | 72.77±0.324 | 65.93±1.47 | 56.68±1.78 | 61.4±1.38  |
| 400          | 77.09±0.462 | 71.77±1.55 | 60.44±1.3  | 67.26±1.17 |
| 500          | 82.56±0.345 | 76.21±1.17 | 64.79±0.77 | 70.43±0.78 |

*Mean (triplicates) ±SD

Fig. 4: Graph showing comparative percent inhibition of protein denaturation activity in different parts of *Aralia cachemirica*

**HRBC Membrane stabilization method**

The result of % inhibition of membrane stabilization method in various parts of A. cachemirica is presented in the table 5 and fig. 5. From the table it is clear, that the leaves show maximum % inhibition of membrane stabilization followed by the root and least inhibition is shown by the stem. Also, from the table it is apparent that as the concentration of the extracts increases the % inhibition of membrane stabilization also shows an increase.

**Table 5: Comparison of % inhibition of membrane stabilization in different parts of *Aralia cachemirica***

| Conc. (µg/ml) | Indomethacin | Plant parts % inhibition |
|--------------|--------------|-------------------------|
## Discussion

Three standard methods viz. DPPH radical scavenging activity, reducing power activity and metal chelating activity are followed for assessing the antioxidant potential of *A. cachemirica*. The results obtained from our study reveal that different part of *A. cachemirica* possesses considerable antioxidant potential thereby suggesting that the plant extracts contain compounds that are excellent hydrogen donors. The results of all the three methods employed for the study of antioxidant activity reveal that among different parts of *A. cachemirica* the maximum antioxidant potential is in the leaves followed by the root and then stem. The findings of different antioxidant assays also depict that free radical scavenging is concentration dependent. Our study is in agreement with the findings of Liu *et al.*, [18] who evaluated antioxidant activity of *Aralia cordata*. Also, Lee *et al.*, [19] determined antioxidant potential of *Aralia elata*.

During the present investigation two standard methods viz. protein denaturation method and HRBC membrane stabilization method are employed to determine anti-inflammatory activity of different parts of *A. cachemirica*. The results obtained for *A. cachemirica* show that the different parts of the plant possess anti-inflammatory activity.
Also, the results reveal that maximum anti-inflammatory activity is shown by leaf followed by the root and then stem. Furthermore, anti-inflammatory activity is in concentration dependent manner. The different plant parts studied show both significant inhibition of protein denaturation and HRBC membrane stabilization activity. Our study corroborates with the findings of Lim et al., [20] who reported anti-inflammatory activity in the roots of Aralia continentalis. Also, Prasanth et al., [21] determined anti-inflammatory activities of methanolic extract of Aralia racemosa root. Moreover, Lee et al., [19] determined anti-inflammatory potential of Aralia elata.

Conclusion

This is the first study providing information regarding antioxidant and anti-inflammatory potential of different parts of the species. The antioxidant activity of the extract could have great importance as therapeutic agents in averting or slowing the advancement of aging and age associated, oxidative stress related degenerative diseases. Plant derived anti-inflammatory agents are competent cure for inflammation caused by a range of agents, which shows the way to their soaring requirement. Exploitation of these agents requires to be regulated in order, to be sustainable and keep up with demands.

Declaration

Ethics approval and consent to participate: The authors Neelofar Majid and Saduf Nissar were themselves the healthy blood donors for the experiment. Since, the authors voluntarily donated blood for the experiment there was no need to get approval from any ethics board/institution. a) All methods were carried out in accordance with relevant guidelines and regulations. b) All experimental protocols were approved by a named institutional and/or licensing committee. c) Informed consent was obtained from all subjects.

Consent for publication: Not applicable.

Availability of data and material: All data generated or analyzed in this study are included in this manuscript.

Competing interests: No conflict.

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Authors' contributions: NM and SN carried the experimental work and result analysis; WYR and SR helped in the compilation of data; IAN and ZAB helped in the supervision of the work. All authors have read and approved the final manuscript.

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