

## SHORT COMMUNICATION

### *In vitro* anti-inflammatory activity of *Amaranthus caudatus* L. leaves

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Present investigation has been undertaken to investigate the anti-inflammatory activity of *Amaranthus caudatus* L. (Family Amaranthaceae) leaves using *in vitro* models. Leaves were extracted with 80 % butanol and methanol, both the extracts produced significant anti-inflammatory activity in concentration dependent manner. Butanol and methanol extract at 500 µg/mL produced 54.10 and 49.33 % inhibition of protein denaturation, respectively; while at same concentration butanol and methanol extract exhibit 78.41 and 70.33 % protection, respectively against RBC membrane degradation.

**Keywords:** *Amaranthus caudatus* L., Anti-inflammatory, Leaves, *In vitro*

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### Introduction

*Amaranthus caudatus* L. (Family Amaranthaceae) is a leafy vegetable, which was acknowledged in different traditional and folk medicinal system for its medicinal value. The plant is traditionally used to cure kidney stones, stomach pain, leprosy, fever, pulmonary conditions and piles. Abortifacient effect of the plant is also described in folk medicine<sup>1,2</sup>. Scientifically the plant has been investigated for antioxidant<sup>3</sup>, antidiabetic and hypoglycaemic<sup>1,3</sup>, hypolipidemic, anti-hypercholesterolemic<sup>2,4,5</sup>, anthelmintic<sup>6</sup> and antimicrobial activity<sup>7,8</sup>.

Inflammation is considered as a normal protective response as a part of host defense system during tissue injury. Inflammatory responses involve release of chemo-attractant mediators and chemo-activators, enzyme activation, tissue break down and repair. But inflammation may also be responsible for serious fatal situation<sup>9</sup>. A number of synthetic drugs particularly non-steroidal anti-inflammatory drugs (NSAIDs) are available for the treatment of

inflammation. But most of them possess serious side effects like gastric or duodenal ulcer, anemia, hepatotoxicity, diarrhoea, abdominal pain, rash, etc. Plant based drugs have lesser side effects and more physiological compatibility<sup>9-12</sup>. Present work aim to investigate anti-inflammatory activity of *A. caudatus* L. leaves using different *in vitro* models.

### Materials and Methods

#### Chemicals

Analytical Grade butanol, methanol, ferric chloride, lead acetate and zinc chloride were obtained from Loba Chemie, SD Fine Chem Ltd. and used for study. All the other reagents used were also of analytical grade.

#### Plant materials

Leaves of *A. caudatus* L. were collected from the Panikahaiti, Guwahati, Assam and duly authenticated by Dr. A A Mao, Scientist-Botanical Survey of India, Shillong, India (Voucher specimen No. BSI/ERC/Tech/Plant Iden./2014/10).

#### Extraction

Leaves of the plant were cleaned to remove unwanted material and dried in shed. Air dried leaves were pulverized to get fine powder and was extracted with 80 % butanol and methanol in Soxhlet apparatus separately. Solvent was evaporated to dryness on water bath to get solvent free methanol extract (yield: 8.4 % w/w) and butanol extract (yield: 6.5 % w/w) of *A. caudatus* L. leaves.

#### Preliminary phytochemical screening

Small amount of extract was investigated to find the presence of different phytochemicals in particular solvent extract. Standard methods were used to determine the presence of phytochemicals like alkaloid, glycoside, tannin, protein, amino acid, steroids and terpenoids<sup>13,14</sup>.

#### Inhibition of protein denaturation

About 0.2 mL of egg albumin (obtained from fresh hen egg), 2.8 mL of phosphate buffer saline (PBS, pH 6.4) and 2.0 mL of different concentrations of extracts of *A. caudatus* L. leaves were taken in test tubes. Final concentration of each extract in 5.0 mL of reaction mixture was 100, 200, 500 µg/mL. Distilled

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water (5.0 mL) served as control. Test tubes were incubated at 37 °C in a BOD incubator for 15 min and then heated at 70 °C for 5 min. Test tubes were cooled and the absorbance was measured at 660 nm using UV Spectrophotometer<sup>15</sup>. Aspirin (100, 200 µg/mL) was used as standard drug and treated similarly to determine the absorbance. Percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times [V_t / V_c - 1]$$

Where,  $V_t$  = absorbance of test sample,  $V_c$  = absorbance of control.

#### Red blood cell membrane stabilizing assay

Blood (5 mL) was collected from healthy adult rat and mixed with 5.0 mL of Alsever solution (2.0 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl in water). Mixture was centrifuged at 3000 rpm and packed cells were washed 3 times to remove cell debris with isosaline (0.85 %, pH 7.2). Erythrocyte suspension (10 % v/v) was prepared using isosaline. Assay mixture contains various concentration of extracts (100, 200 and 500 µg/mL), 1.0 mL phosphate buffer (0.15 M, pH 7.4), 2.0 mL hyposaline (36 %) and 0.5 mL red blood cell suspension. The mixture was incubated at 37 °C for 30 min and then centrifuged at 3000 rpm for 20 min. Hemoglobin content of the supernatant was determined using spectrophotometer at 560 nm<sup>16</sup>. Aspirin (100 and 200 µg/mL) was used as standard and the control was prepared by omitting the extract. Percentage of RBC membrane stabilization was estimated by following formula:

$$\% \text{ protection} = 100 \times [(OD_1 - OD_2) / OD_1]$$

$OD_1$  = Optical density of drug treated sample,  
 $OD_2$  = Optical density of control

#### Statistical analysis

All the experiments were done in triplicates and the results were expressed as Mean±SEM. The inhibition of specific concentration was calculated using specific formula.

#### Results and Discussion

Methanol and butanol extracts of *A. caudatus* L. leaves were studied for *in vitro* anti-inflammatory activity by RBC membrane stabilization and inhibition of protein denaturation method. Qualitative phytochemical investigation revealed that methanol extract contains alkaloids, glycosides, tannins,

flavonoids, steroids and triterpenoids, while saponin, tannins, flavonoids, steroids and triterpenoids were present in butanol extract. Results obtained from the study demonstrate that extracts inhibited protein denaturation and RBC haemolysis in concentration dependent manner. At highest concentration butanol extract produced 54.10 % inhibition of protein denaturation and methanol extract produce 49.33 % inhibition (Table 1). Both the extracts exhibited significant membrane stabilizing effect, at 500 µg/mL butanol and methanol extract produced 78.41 and 70.33 % protection, respectively against RBC degradation while standard aspirin (200 µg/mL) produced 85.04 % protection (Table 2).

These types of *in vitro* methods are important to know the role of chemical mediators and anti-inflammatory mechanism<sup>17</sup>. Both butanol and methanol extracts produced membrane stabilization effect by inhibiting lysis of erythrocyte membrane caused by hypotonicity. Erythrocyte membrane is comparable with that of the lysosomal membrane. Stabilization of RBC membrane implies that the

Table 1—Influence of *A. caudatus* L. leaves extracts against protein denaturation

Extract/Standard	Concentration (µg/mL)	% Inhibition
Aspirin	100	41.33 ± 1.09
	200	55.24 ± 2.41
	100	15.04 ± 0.99
Butanol extract	200	35.22 ± 1.70
	500	54.10 ± 2.04
	100	15.88 ± 1.11
Methanol extract	200	34.02 ± 2.00
	500	49.33 ± 2.55

Results are expressed as mean±SEM (n=3)

Table 2—Red blood cell stabilizing effect of *A. caudatus* L. leaves extracts

Extract/Standard	Concentration (µg/mL)	% Protection
Aspirin	100	60.11 ± 2.01
	200	85.04 ± 3.01
	100	28.34 ± 1.23
Butanol extract	200	62.09 ± 2.77
	500	78.41 ± 2.99
	100	20.09 ± 1.02
Methanol extract	200	57.03 ± 3.07
	500	70.33 ± 2.09

Results are expressed as mean±SEM (n=3)

extracts are capable to stabilize lysosomal membranes. This feature is considered as important step to limit the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil, which may induce tissue inflammation and damage upon extracellular discharge<sup>18</sup>. Thus a possible mechanism for anti-inflammatory activity of *A. caudatus* L. may be due to the inhibitory action on release of inflammatory mediators responsible for inflammation through membrane stabilizing effect. In inhibition of protein denaturation method egg albumin denaturation was induced by heat. Denatured protein due to heat treatment expresses antigens related to type III hyper-sensitive reaction. These denatured proteins are equally effective as native proteins and play a key role to provoke delayed hypersensitivity. Denaturation of protein is considered as one of the reasons of inflammation. Auto-antigen production in inflammatory diseases like rheumatoid arthritis may be due to denaturation of protein in *in vivo* condition. Alteration of electroelastic, hydrogen, hydrophobic and disulphide bonding can be related to denaturation of protein. A number of NSAIDs like phenylbutazone and indomethazine also prevent denaturation of proteins, along with inhibition COX enzyme<sup>17,19</sup>. The result of the study clearly had shown that *A. caudatus* L. extracts are capable to modify the synthesis of auto antigen and thus inhibits the protein denaturation. Secondary metabolites like alkaloids, glycosides, phenolic compounds and tannins which were found in qualitative phytochemical screening might be responsible for the observed activity.

### Conclusion

Based on the present study it can be concluded that the methanol and butanol extract of *A. caudatus* L. leaves possess *in vitro* anti-inflammatory activity which might be attributed to the presence of various phytochemicals in the extract. Membrane stabilizing effect and the protein denaturation activity of extract may be responsible for the anti-inflammatory activity. However, further investigations are essential to isolate the active constituents and to investigate the exact anti-inflammatory mechanisms of these leaves.

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