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# *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACT OF *ARISTOLOCHIA BRACTEOLATA*

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## ABSTRACT

*Aristolochia bracteolata* is an important medicinal herb and it is an original of Indian subcontinent and has become naturalized in the tropical and sub-tropical areas around the world. The plant is usually gathered from the wild and is used locally in traditional medicine. It is sometimes cultivated for medicinal use in India. In the indigenous system of medicine, the plant was used as purgative, antipyretic & anti-inflammatory agents. The root part has antifungal and antibacterial activity and was used to treat syphilis, gonorrhoea, and skin diseases and also used during labours to increase uterine contraction. Its leaves are bitter and anti-helmintic, antiulcer, anti-plasmodial and are medicinally important. The whole plant is very bitter and has abortifacient, alterative, anthelmintic, antiperiodic, emmenagogue and purgative properties. In the current investigation, Leaf extract of *Aristolochia bracteolata* has shown anti-inflammatory and antioxidant properties. *Aristolochia bracteolata* ABETpowder extract has shown significant in vitro antioxidant activity by DPPH assay in the current investigation. At 100 mg/ml ABET, the average inhibition percentage is 67%, with the $IC_{50}$ value was found to be 216.7884 mg/ml compared to the standard ascorbic acid $IC_{50}$ value of 33.7334 mg/ml. Further investigation of in vitro anti-inflammatory activity by the protein denaturation method compared to the standard. At 100 mg/ml, ABET has an average inhibition percentage of 40%, with the $IC_{50}$ value was found to be 296.1292 mg/ ml compared to the standard $IC_{50}$ value of 121.29 mg/ml.Overall investigation results, such as ABET, had good antioxidant and moderate anti-inflammatory activity.

**KEYWORDS:** *Aristolochia bracteolata*, antioxidant, DPPH assay, anti-inflammatory.

## INTRODUCTION

*Aristolochia indica* L. of the family Aristolochiaceae is a twining perennial herb with an ancient history of medicinal use. In the Indian Ayurvedic system, it is used for the treatment of snake venom, scorpion venom, pimple, fever and worm infections (Padhy, G.K., 2021). *Aristolochia bracteolata* is a perennial herb, the leaves of which are used by the native tribals and villagers of the Chittoor District of Andhra Pradesh in India for the rapid healing of cuts and wounds. The ethanol extract of the shade-dried leaves of *Aristolochia bracteolate* Lam. was studied for its effect on wound healing in rats, using incision, excision and dead-space wound models (Shirwaikar, A., et al, 2003). A small glabrous shrub or a perennial herb, that grows in India and found in Africa, especially the Sahel region from Mali to northern Nigeria, tropical East Africa, and in Saudi Arabia. All parts of the plant are extremely bitter, purgative, emmenagogue, anthelmintic, alterative and antiperiodic. Leaves are administered with castor oil as a remedy for colic, and the juice of fresh leaves or powder of dried leaves is a favorite application for sores, obstinate itch, and to destroy maggots. Root and leaves yield a thick yellowish juice, which is mixed with boiled milk and used in cases of syphilis; and combined with opium it is used for gonorrhea with great success. The plant increases or induces uterine contractions, and hence used in difficult labor, dysmenorrhea and amenorrhea. Its anthelmintic property is also doubtless, and in Africa it is mostly used in veterinary practice. It is indigenous to central Sudan, and medicinally used for its analgesic and diuretic effects, treatment of tumors, malaria and/or fevers; other uses in Sudanese folk medicine include, leaves for the treatment of malaria, and the roots as anti-inflammatory, and for scorpion stings (Akbar, S., 2020). Aristolochic Acids (AAs) are major components of plants in Aristolochia and have been found to be nephrotoxic, carcinogenic and mutagenic. Herein reported are the isolation, identification and quantity determination methods of Aristolochic Acid-I (AA-I) and Aristolochic Acid-II (AA-II) toxic compounds of Aristolochia bracteolata indigenous to *Central Sudan and medicinally used in diverse biological functions including analgesic and diuretic effects, treatment of tumors, malaria and/or fevers (*Abdelgadir, A.A., Ahmed, E.M. and Eltohami, M.S., 2011).Raju, M.G. and Reddy, T.H.S., 2017to evaluate the antidiabetic and hypolipidemic activity of methanolic extract of whole plant of Aristolochia bracteolata (MEAB) against streptozotocin (STZ) induced diabetic rat model. The effect of *Aristolochia bracteolata* extract on Human dermal fibroblast (HDF) and Human keratinocyte cell line (HaCaT) proliferation and migration during *in vitro* wound healing and its underlying mechanism(Girija, D.M., et al, 2017). The treatment with the plant extract and glibenclamide caused marked decreased in the blood glucose level and improved the heamatological indices. The results further showed a marked decrease in ALT, AST and ALP activity in the treatment groups when compared with the positive control group (Group 5). These findings showed that A. bracteolata possess potent antidiabetic properties and beneficial in the management of Type 1 diabetes (Gbadamosi, I.T., Adeyi, A.O. and Owoye, O., 2018.). Methanol extract of *Aristolochia bracteolata* whole plant was carried out in order to evaluate its antimicrobial activity and to identify the active compounds in this extract. Antibacterial and antifungal activities of methanol extract against gram-positive, gram-negative, and fungal strains were investigated by the agar disk diffusion method (Mohamed, M.S., et al 2014). he *Aristolochia bracteata* is well known for its antiarthritis properties in Indian system of medicine and folk medicine(Chitme, H.R. and Patel, N.P., 2009).*Aristolochia bracteolata*. Aqueous, methanol and chloroform extracts of this plant were evaluated against the bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Shigella flexneri*, *Proteus vulgaris* and the fungal strains like *Aspergillus niger*, *Aspergillus terreus*, *Penicillium notatum* and *Rhizopus stolonifer* (Kavitha, D. and Nirmaladevi, R., 2009. ). Das, T.S., Ramesh, L. and Agastian, P., 2016 to evaluate the antioxidant activity of hexane, ethyl acetate and methanol extracts of *Aristolochia bracteolata* Linn. Hexane, ethyl acetate and methanol extract were prepared

using soxhlet extraction. DPPH and GC-MS were also performed.Based on the above facts, the present work the leaf extract of *Aristolochia bracteolate* and their potential investigate their pharmacological explanations for their the in vitro antioxidant activity by DPPH assay compared to the standard ascorbic acid vitamin C and the anti-inflammatory activity by the protein denaturation method compared to the standard diclofenac sodium.

## MATERIAL AND METHODS

### Collection of samples

*Aristolochia bracteolate* powdercollected from Madurai, Tamilnadu for this investigation.

### Method of preparation of samples

10 gram of Aristolochia bracteolate powder is heated with 100 ml of ethanol and separately for 5 hours under reflux condenser in water bath, cool and filter. The filtrate is evaporated under vacuum to get ethanolic extract (ABET).

### Pharmacological evaluation

### In vitro ABET Antioxidant activity

*Aristolochia bracteolate* **(ABET)** aqueous extract investigated for in vitro antioxidant activity by DPPH, ABTS, FRAP and NO for the estimation of anti-oxidant potential of *Aristolochia bracteolate* **(ABET)** aqueous extract. *Aristolochia bracteolate* **(ABET)** me also tested by DPPH assay.

### Determination of DPPH radical scavenging activity

Antioxidant activity in the sample ***Heart spl*** were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (Brand-Williams et al., 1995). 100µL of SC extract was taken in the microtiter plate. 100µL of 0.1% Methanolic DPPH was added to the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. Read the plate on Elisa plate reader at 490nm. Standard ascorbic acid was used as reference. All the analysis was performed in triplicates and the average values were taken.

### Radical scavenging activity was calculated by the following equation

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100.

### ABET In vitro antioxidants activity by DPPH assay

**100mg n=6- ABET**

| S. No. | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.31 | 0.09 | 70% | |
| 2 | 0.31 | 0.10 | 67% | |
| 3 | 0.31 | 0.13 | 58 % | **67%** |
| 4 | 0.31 | 0.08 | 74% | |
| 5 | 0.31 | 0.09 | 70% | |
| 6 | 0.31 | 0.11 | 64% | |

**200mg n=6 - ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.31 | 0.18 | 41% | |
| 2 | 0.31 | 0.18 | 41% | |
| 3 | 0.31 | 0.17 | 45 % | **48%** |
| 4 | 0.31 | 0.17 | 45% | |
| 5 | 0.31 | 0.16 | 48% | |
| 6 | 0.31 | 0.15 | 51% | |

**300mg n=6 - ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.31 | 0.20 | 35% | |
| 2 | 0.31 | 0.21 | 32% | |
| 3 | 0.31 | 0.22 | 29 % | **32.5%** |
| 4 | 0.31 | 0.21 | 32% | |
| 5 | 0.31 | 0.19 | 38% | |
| 6 | 0.31 | 0.22 | 29% | |

**400mg n=6ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.31 | 0.15 | 25% | |
| 2 | 0.31 | 0.17 | 29% | |
| 3 | 0.31 | 0.21 | 19 % | **24.5%** |
| 4 | 0.31 | 0.20 | 25% | |
| 5 | 0.31 | 0.18 | 29% | |
| 6 | 0.31 | 0.19 | 22% | |

**500mg n=6ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.31 | 0.19 | 19% | |
| 2 | 0.31 | 0.17 | 22% | |
| 3 | 0.31 | 0.17 | 25 % | **22%** |
| 4 | 0.31 | 0.19 | 19% | |
| 5 | 0.31 | 0.21 | 22% | |
| 6 | 0.31 | 0.18 | 25% | |

**Standard Ascorbic acid VIT C**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.31 | 0.02 | 93% | |
| 2 | 0.31 | 0.02 | 93% | **93%** |
| 3 | 0.31 | 0.02 | 93 % | |

**STANDARD ASCORBIC ACID $IC_{50}$ VALUE (mg/ml)**

| S. No | Concentration (mg) | Average (%) | $IC_{50}$ value (mg/ml) |
|---|---|---|---|
| 1 | 50 | 91% | |
| 2 | 100 | 87% | |
| 3 | 150 | 86% | 33.7334 mg/ml |
| 4 | 200 | 92% | |
| 5 | 250 | 84% | |

**ABET Antioxidant $IC_{50}$ value (mg/ml)**

| S. No | Concentration (mg) | Average (%) | $IC_{50}$ value (mg/ml) |
|---|---|---|---|
| 1 | 100 | 67% | |
| 2 | 200 | 48% | |
| 3 | 300 | 32.5% | **216.7884mg/ml** |
| 4 | 400 | 24.5% | |
| 5 | 500 | 22% | |

***In-vitro* ABET Anti-inflammatory activity - Inhibition of albumin denaturation**

The reaction mixture was prepared separately by mixing 0.5ml aqueous extract of ABET and its compounds A, B, and C (1mg/ml) with 0.45 ml aqueous solution of bovine albumin fraction (5%). The pH (6.3) of the solution was adjusted using a small amount of 0.1N HCl at 37 ˚C for 20 min, then heat to 57 ˚C for 30 min. Cool the solution and transfer it to the 96 well plates and measure the absorbance at 660nm. Standard was used as Diclofenac sodium (1000µg/ml) and the control contain 0.05ml distilled water. The percentage of inhibition of albumin denaturation was calculated by the following formula, Percentage of inhibition (%) = [(A control – A sample) / A control] x 100 Where A control – Absorbance of reaction mixture except drug. A sample – absorbance of the reaction mixture with the Sample.

**ABET *In vitro* Anti inflammatory activity by the protein denaturation**

**100mg n=6- ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.37 | 0.25 | 32% | |
| 2 | 0.37 | 0.23 | 37% | |
| 3 | 0.37 | 0.20 | 45 % | **40%** |
| 4 | 0.37 | 0.20 | 45% | |
| 5 | 0.37 | 0.21 | 43% | |
| 6 | 0.37 | 0.22 | 40% | |

**200mg n=6 - ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.37 | 0.21 | 43% | |
| 2 | 0.37 | 0.22 | 40% | |
| 3 | 0.37 | 0.22 | 40 % | **39%** |
| 4 | 0.37 | 0.22 | 40% | |
| 5 | 0.37 | 0.23 | 37% | |
| 6 | 0.37 | 0.24 | 35% | |

**300mg n=6 - ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.37 | 0.24 | 35% | |
| 2 | 0.37 | 0.25 | 32% | |
| 3 | 0.37 | 0.26 | 29 % | **34%** |
| 4 | 0.37 | 0.24 | 35% | |
| 5 | 0.37 | 0.23 | 37% | |
| 6 | 0.37 | 0.23 | 37% | |

**400mg n=6ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.37 | 0.28 | 24% | |
| 2 | 0.37 | 0.26 | 29% | |
| 3 | 0.37 | 0.27 | 27 % | **29%** |
| 4 | 0.37 | 0.25 | 32% | |
| 5 | 0.37 | 0.25 | 32% | |
| 6 | 0.37 | 0.25 | 32% | |

**500mg n=6ABET**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.37 | 0.25 | 32% | |
| 2 | 0.37 | 0.26 | 29% | |
| 3 | 0.37 | 0.25 | 32 % | **31.5%** |
| 4 | 0.37 | 0.26 | 29% | |
| 5 | 0.37 | 0.24 | 35% | |
| 6 | 0.37 | 0.25 | 32% | |

***In vitro* anti-inflammatory activity compared to standard Diclofenac sodium**

| S. No | COD | SOD | %inhibition | Average |
|---|---|---|---|---|
| 1 | 0.37 | 0.07 | 81% | |
| 2 | 0.37 | 0.07 | 81% | **81%** |
| 3 | 0.37 | 0.07 | 81% | |

**Standard diclofenac sodium $ic_{50}$ value (mg/ml)**

| S. No | Concentration (mg) | Average (%) | $IC_{50}$ value(mg/ml) |
|---|---|---|---|
| 1 | 50 | 91% | |
| 2 | 100 | 93% | |
| 3 | 150 | 85% | 121.29 mg/ml |
| 4 | 200 | 89% | |
| 5 | 250 | 88% | |

**ABET Anti-inflammatory $IC_{50}$ Value (mg/ml)**

| S. No | Concentration (mg) | Average (%) | $IC_{50}$ Value (mg/ml) |
|---|---|---|---|
| 1 | 100 | 40% | |
| 2 | 200 | 39% | 296.1292mg/ml |
| 3 | 300 | 34% | |
| 4 | 400 | 29% | |
| 5 | 500 | 31.5% | |

## RESULT AND DISCUSSION

Ethanolic extract of *Aristolochia bracteolate* has shown anti-inflammatory and antioxidant properties. *Aristolochia bracteolate* ABET powder extract has shown in vitro antioxidant activity by DPPH assay in the current investigation. At 100 mg/ml ABET, the inhibition percentage is 67%, and the $IC_{50}$ value was found to be 216.79 mg/ml compared to the standard ascorbic acid $IC_{50}$ value of 33.73 mg/ml. As a result, even a low concentration of antioxidant activity is good compared to the standard ascorbic acid vitamin C. A previous paper reported that in vitro antioxidant activity using seven different methods. The successive ethyl acetate and crude methanol extracts have shown potent antioxidant activity in ABTS method with $IC_{50}$ values of 17.08 mg/ml and 28.12 mg/ml, respectively. The inhibition of the albumin denaturation method was used to measure the anti-inflammatory activity in vitro. In comparison to standard diclofenac sodium, neither the crude extract ABET nor its separated constituents exhibit any appreciable anti-inflammatory

efficacy. When compared to standard diclofenac sodium, the aqueous extracts of ABET exhibit moderate anti-inflammatory efficacy. At 100 mg/ml, ABET had a 40% with $IC_{50}$ value of 296.1292 mg/ml compared to the standard diclofenac sodium $IC_{50}$ value of 121.29 mg/ml. While we were carrying out different concentrations in a dose-dependent way and comparing them with the standard, many of them showed the antioxidant and anti-inflammatory properties of ABET. A previous investigation scientifically validates the use of *A. bracteolata* in wound healing (Girija, D.M.et al 2017. Overall investigation results, such as ABET, had good antioxidant and moderate anti-inflammatory activity.

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