



ISSN Print: 2664-9926

ISSN Online: 2664-9934

IJBS 2025; 7(1): 05-09

www.biologyjournal.net

Received: 07-10-2024

Accepted: 12-11-2024

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A comparative evaluation of enzymatic antioxidant and anti-inflammatory properties of *Acmella uliginosa* (Sw.) Cass. and *Blumea sinuata* (Lour.) Merr

Deepthi Rawat and PB RaoDOI: <https://dx.doi.org/10.33545/26649926.2025.v7.i2a.289>**Abstract**

The growing area of research in immune metabolism reveals that metabolic reactions not only generate ATP and precursor biomolecules for other biosynthetic pathways, they are also generating free radicals. Once these free radicals are produced in the cell, to get stabilized, they react with different biomolecules such as lipid, protein, and nucleic acid, causing various diseases such as cancer, diabetes mellitus, cardiovascular diseases, atherosclerosis, etc. Antioxidant-rich medicinal plants are a possible source for the evolution of the management of health care and pharmaceutical industries. In the present study, we aimed to evaluate the enzymatic antioxidant and anti-inflammatory properties of two selected plant species of the family Asteraceae. Antioxidant properties were investigated, by using superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) enzymatic activities. The highest SOD, POD, and CAT activities showed by *Blumea sinuata* 18.23 SOD U/ mg of fresh weight, 108.58 nmol/mg of protein, and 40.33 $\mu\text{mol/min/mg}$ of protein respectively. The highest anti-inflammatory activity in aqua methanol exhibited by *A. uliginosa* and in acetone extract was slightly higher in *B. sinuata*.

Keywords: Enzymatic antioxidant, anti-inflammatory, *Acmella uliginosa*, *Blumea sinuata*, free radicals

Introduction

Free radicals which are derivatives of oxygen, nitrogen, and sulfur are produced in the human body via various endogenous systems (Such as different metabolic reactions) as well as on exposure to pathophysiological states. These radicals have been involved in the etiology of various threatening ailments and aging by adversely altering the functions of lipid, protein, and genome of the organism [9]. But these free radicals also play an important role as a signaling molecule and maintain body homeostasis at the cellular level [31]. Antioxidants are those biomolecules that neutralize the action of free [29]. In nature, two different categories of antioxidants exist i.e. enzymatic and nonenzymatic. Enzymatic antioxidants are superoxide dismutase (SOD), peroxidase (POD), glutathione reductase, thioredoxin, and catalase (CAT), while phenols, flavonoids, carotenoids, ascorbic acid (vitamin C), and α -tocopherol (vitamin E) are nonenzymatic antioxidants. To diminish the detrimental effects of these free radicals, enzymatic antioxidants play a crucial role [30].

It has been validated that the administration of superoxide dismutase and catalase, are showing positive results at attenuating various diseases related to tissue inflammation such as arthritis, chronic gut inflammation, reperfusion, ischemia, and immune complex induced pulmonary injury [6]. These are the first detoxification and most powerful endogenous enzyme in the cell, act to defend cells from reactive oxygen species. Superoxide dismutase (SOD) required a metal as a cofactor for its action, based on their metal requirement, various form of SOD metalloenzymes are present such as (1) Fe-SOD, enzyme bounded by iron, present in prokaryotes and chloroplast of plant cells (2) Mn-SOD in this enzyme surrounded by magnesium, reported in prokaryotes and mitochondria of eukaryotes and (3) Cu/Zn SOD, an enzyme required a copper or zinc as a bivalent cofactor for its activity and reported in the cytosol of the most of eukaryotes [11, 19]. Peroxidase (POD), that breakdown reduces hydrogen peroxides (H_2O_2) into the water while oxidizing a variety of substrates [10].

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It protects the cell from oxidative stresses by hindering the peroxidation of lipid. Catalase is a tetra homomeric protein with mol weight 240 kDa, encoded by *cat* gene, and finished the detoxication process introduced by superoxide dismutase. CAT is a very common antioxidant enzyme which required iron or sometimes manganese as a cofactor for the reduction of hydrogen peroxide to water and molecular oxygen [15]. Reactive oxygen species are also responsible for promoting the initiation or amplification of inflammation by the up-regulation of different genes participated in the inflammatory response. Inflammation is an immunological process, associated with redness, swelling, pain, stiffness of muscles, and fever, in which leukocytes and their output products such as vasodilators (histamines) implemented immunity to the human body. It is a substantial factor for the progress of pathological invaders in the vital organs, inflammation is a frequent pathogenesis response for various chronic diseases such as cancer, hyperglycemia, autoimmune and cardiovascular diseases, and it is activated in coordinated signaling pathways [22].

In humans, autoimmune diseases, rheumatoid arthritis along with Psoriatic arthritis and Gouty arthritis are associated with inflammation reaction. Microbial products, cytokines such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) are immediate inflammatory provocations, they initiated inflammatory reactions by interaction with the TLRs, IL-1 receptor (IL-1R), IL-6 receptor (IL-6R), and the TNF receptor (TNFR) [17]. Once stimuli interact with their receptors, an intracellular signaling pathway gets activated such as nuclear factor kappa-B (NF- κ B), mitogen-activated protein kinase (MAPK), and Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathways [5].

In the present investigation, *A. uliginosa* and *B. sinuata* two herbaceous plant species of the family Asteraceae were collected for evaluation of their anti-inflammatory properties in two different solvent extracts (aqua acetone and aqua methanol). Taxa *Acmella* with 30 species distributed in the tropical and subtropical regions all over the world. It exhibited various ethnomedicinal importances, commonly associated with the treatment of throat pain, stomachache, mouth ulcer, and toothache, spilanthol is an alkaloid that was reported to be related to these activities [33]. Previous studies revealed the presence of numerous bioactive compounds, terpenes glycosylated, anthracene derivatives, coumarins glycosylated, glycosylated flavonoid, anthracene derivatives, alkaloids, essential oils, tannins, and saponins in *A. uliginosa* [21, 24]. Members of Asteraceae have been identified for the production of polyphenols, flavonoids and sesquiterpenoids secondary metabolites in their vegetative as well as in reproductive parts [8].

Materials and Methods

Plant materials

Plant species *Acmella uliginosa* and *Blumea sinuata* were collected from the campus of G.B Pant University, Pantnagar (29.0222° N, 79.4908° E), samples were air-dried, then ground to a fine powder in an electrical grinder.

Preparation of extracts

Plant material (10 gm dried) was extracted with 100 ml of 80% aqua acetone and aqua methanol in the rotatory shaker at 120 rpm for one week at room temperature. The extracted sample was filtered with the help of the Whatman filter

paper, after filtration, the supernatant was again preceded to the centrifugation process. Then it was transferred to Petri-plate for evaporation of the respective solvent. The extracts obtained after the evaporation of solvents were kept at -4°C until an evaluation of the anti-inflammatory property.

Protein estimation

Fresh plant material was used for quantification of protein and evaluated according to the method previously described by [23] with the required modifications.

Superoxide dismutase (SOD) assay

The ability of superoxide dismutase (SOD, EC 1.15.1.1) was determined by using the method reported by [12]. The reaction mixture was prepared by 50 mM phosphate buffer (pH=7.8), 13 mM methionine, 0.1 μ M EDTA, 2 μ M riboflavin, 75 μ M NBT (nitro blue tetrazolium), and 100 μ l crude enzyme. A control was prepared without the addition of crude extract. The enzyme mixture spectrometrically measured at 560 nm for measuring superoxide dismutase (SOD, 1 unit) responsible for photo-reduction of NBT by 50% with the following formula:

$$Z=(X-A) \times 100$$

Where Z= photo-inhibition % of the sample; X= absorbance of control; A= absorbance of enzyme sample; and Z/50= total SOD unit.

Peroxidase (POD) assay

Peroxidase activity (POD, EC 1.11.1.7) was analyzed by methodologies proposed by [18, 27] with the required medications. The reaction is based on H₂O₂ decomposition into H₂O and O₂ via peroxidase in the presence of a dye such as o-dianisidine or pyrogallol (hydrogen donor). The increasing absorbance of colored oxidized products, i.e., purpurogallin is measured by optical density (Spectrometrically) at 470 nm as a 1POD unit. One unit of POD was determined as 1mg/ml pupurogallin formed from pyrogallol in 60 sec at 6.5pH and measured by the following formula:

$$U/ml \text{ enzyme} = (At/min - Ao/min) \times 12 \times 0.1 \times 3 \times df$$

Where, At =Absorbance of test sample; Ao =Absorbance of control; 3 = Volume (ml) of reaction mixture; df = dilution factor; 12 = Extinction coefficient of 1 mg/ml of pupurogallin; and 0.1 = Volume (ml) of the test sample.

Catalase (CAT) assay

Catalase activity (CAT, EC 1.11.1.6) was assayed by the procedure of [18] with suitable modifications by monitoring the breakdown of H₂O₂ by decrease absorbance at 240 nm. The activity is computed by the extinction coefficient (40 mM-1 cm-1) for H₂O₂. Mixture for the reaction was prepared by the addition of 200 M potassium phosphate buffer (pH 7.0), 100 mM sodium phosphate buffer (pH 6.8), 0.005 M H₂O₂ (fresh), and 5% H₂SO₄. A control was prepared without crude extract.

Evaluation of *in vitro* anti-inflammatory activity

An *in vitro* anti-inflammatory assay was evaluated by applying [4] method. The principle behind this assay is to determine to decrease albumin turbidity (protein

denaturation) with increasing plant extract concentration. Fresh phosphate buffer saline (PBS) was prepared by mixing NaCl (8 g), KCl (0.2 g), Na_2HPO_4 (1.44 g), and KH_2PO_4 (0.24 g) in distilled water (up to 1L) (6.4 pH). 1 ml plant extract of suitable concentrations of 50, 100, 150, 200, 250, and 300 $\mu\text{g}/\text{ml}$ was reacted with 0.1 ml egg albumin and 1.9 ml of phosphate buffer saline (PBS, pH 6.4). The reaction mixture was incubated at 37 °C for 15 min and then at 70 °C for 5 min. Diclofenac sodium was taken as a positive standard. After incubation absorbance was measured at 660 nm.

Data presentation and analysis

The findings were expressed as mean and standard error (mean $\pm$ SE), calculated from triplicate and the linear relationship was visually calculated. The significant differences among different groups of data were evaluated with ANOVA by using SPSS 16.0 and Duncan's multiple range tests; the level of significance was $P < 0.05$.

Result

Extraction yield

Leaves of plants were used for the extraction in two different solvents aqua methanol and aqua acetone at room temperature (Table 1). *B. sinuata* gave 15.15 ± 0.20 yield in aqua methanol which is higher than *A. uliginosa* (10.99 ± 0.08). Similarly in aqua acetone, it was again higher in *B. sinuata* (23.09 ± 0.25) as compare to *A. uliginosa* (9 ± 0.57). Extraction is a very crucial step; the extraction yield of the plant depends upon the method of extraction, temperature, pH, time duration, and the polarity of the used solvent.

Protein content

Protein is a result of transcription and translation (central dogma of life); it is a very crucial bio-molecule for the building, maintaining, and repairing of the cell or tissues in the organism. It contributes in defense against biotic as well as abiotic stresses, channels, or pumps for the movement of different ions across the membrane, and the plant also stores protein in seeds, for the production of energy [16]. The result shows protein content in the selected plant species *A. uliginosa* (10.19 ± 0.15) and *B. sinuata* (27.45 ± 0.17) (Fig.1).

Superoxide dismutase activity

Superoxide dismutase (SOD), a metalloenzyme scavenge different reactive oxygen (ROS) by catalyzing the conversion of superoxide anion ($\text{O}^{\bullet-2}$) into oxygen (O_2) and hydrogen peroxide (H_2O_2) [7]. In the present investigation, *B. sinuata* (18.23 ± 0.11 SOD (U/ mg of fresh weight showed higher SOD activity as compared to *A. uliginosa* (8.85 ± 0.14 SOD (U/ mg of fresh weight) (Fig. 2). Previous investigation in different plant species of family Asteraceae reported by different scientist and research scholars. SOD activity in *Echinacea purpurea* (83.78 EU g leaf⁻¹) reported by [14]. In other investigation the SOD activity of methanol extract (57.34 ± 3.21 $\mu\text{g}/\text{ml}$) and chloroform extract (70.2 ± 4.56 $\mu\text{g}/\text{ml}$) of *Sonchus asper* [20]; the whole plant of *Evolvulus alsinoides* (49.8 ± 0.13 U/mg protein) and *Cynodon dactylon* (207.70 ± 3.48 U/mg protein) [13]. These results are comparatively higher than the present study values.

Peroxidase activity

Peroxidase (POD) reduces H_2O_2 into the water [10]. The highest antioxidant enzyme was determined in *B. sinuata* (108.58 ± 0.25 nmol/mg of protein), on the other hand in *A. uliginosa* (75.04 ± 0.23 nmol/mg of protein) (Fig. 3). Enzymatic antioxidant property of different medicinal plants of the family Asteraceae, among them they reported the highest POD activity in *Santolina chamaecyparissus* (415.22 EU g leaf⁻¹) and lowest in *Înula helenium* (57.68 EU g leaf⁻¹) [14]. Methanol extracts of *S. asper* peroxidase activity 67.34 ± 3.21 $\mu\text{g}/\text{ml}$ [20] in *Tylophora pauciora* whole plant (381.57 ± 2.19 U/mg protein) [33]; and in 2016 Alici and Arabaci [1] in *Rumex obtusifolius* (195.24 ± 0.0044 U/ml) are much higher than the present study values.

Catalase activity

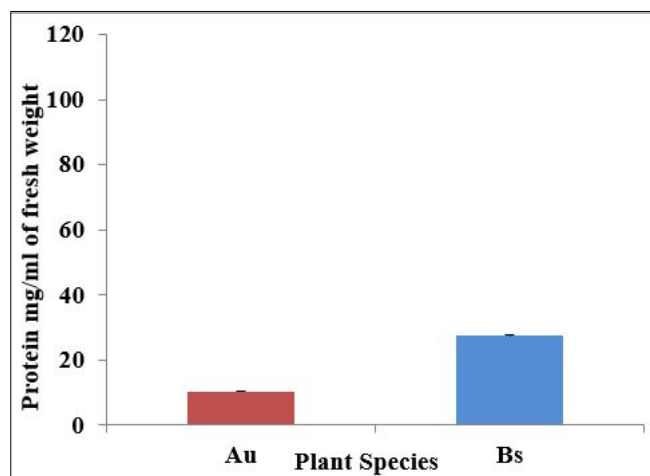
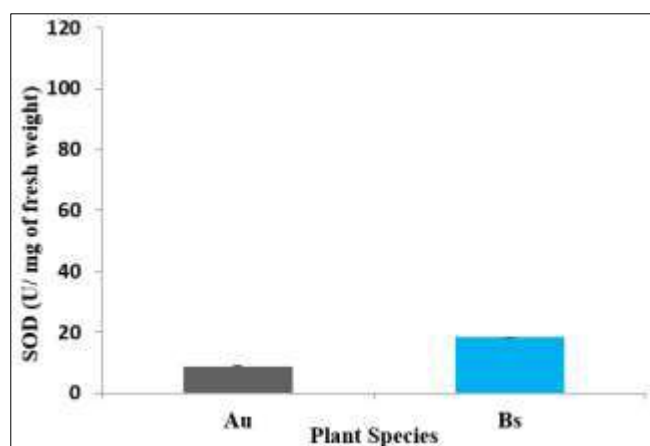
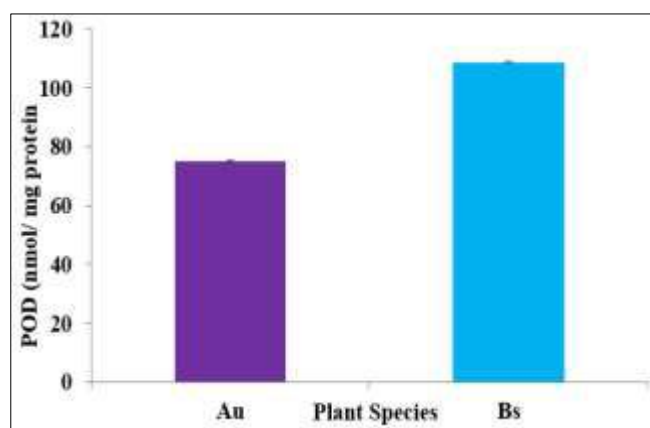
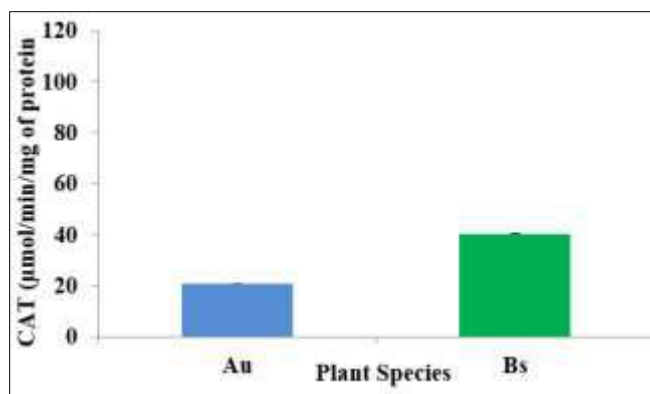
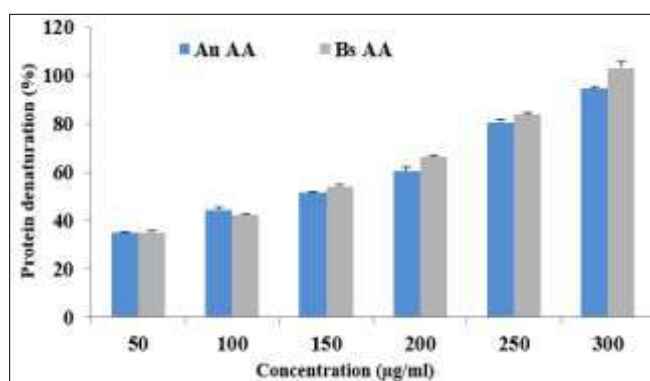
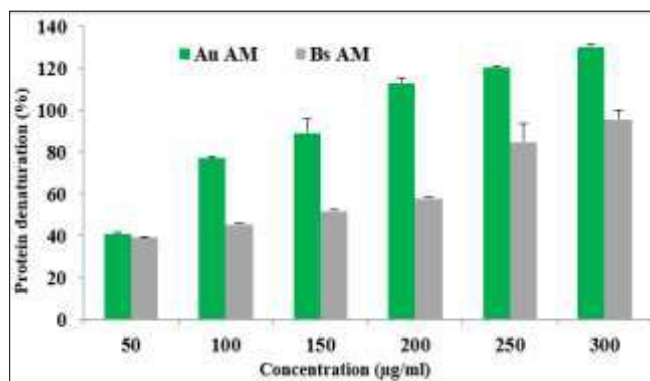
Catalase is a homotetramer enzyme and catalyzes the reaction which is initiated by superoxide dismutase. It catalyzes the breakdown of H_2O_2 (hydrogen peroxide) into H_2O (Water) and O_2 (Oxygen) in a minute [25]. The enzymatic antioxidant activity of selected plant species of Asteraceae was showed a significant difference, the CAT activity of *B. sinuata* (40.33 ± 0.16 $\mu\text{mol}/\text{min}/\text{mg}$ of protein) was higher than the *A. uliginosa* (20.76 ± 0.05 $\mu\text{mol}/\text{min}/\text{mg}$ of protein) (Fig. 4). Gunes *et al.* (2019) [14] reported the enzymatic activity of another Asteraceae member such as in *Santolina chamaecyparissus* L. (1985.08 EU g leaf⁻¹) and *Echinacea purpurea* (522.90 EU g leaf⁻¹). In other plants such as in *Cynodon dactylon* (1.38 ± 0.04 U/mg protein) and *Pongamia glabra* (39.46 ± 0.27 U/mg protein) [26]; in *Tylophora pauciora* whole plant (39.87 ± 0.51 U/mg protein) [33]; and in *Rumex obtusifolius* leaves (38.65 ± 0.00026 U/ml) [1] were more or less similar as compared to the present study values.

In-vitro anti-inflammatory or Protein denaturation

COX-1, COX-2, and 5-LOX are the main enzymes that participate in inflammatory processes, a relatively high amount of biologically active compounds are present in the family Asteraceae display anti-inflammatory properties [3]. In the present study both the plant species shows the anti-inflammatory properties, in the aqua methanol and aqua acetone extracts, in methanol extract it was highest in *A. uliginosa*, and in acetone extract higher in *B. sinuata* (Fig. 5). In 2013 Zheng *et al.* [32] evaluated anti-inflammatory activities of different extracts of *Gnephaliun stramonium* n-hexane (200 mg/kg), methanol (140 mg/kg), and water (no significant anti-inflammatory activity); the anti-inflammatory potential of methanol and acetone extract of *Ximenia americana* leaf, which was (77.51 ± 2.25) and (28.48 ± 2.11) respectively [28]. In 2015 Chagas-Paula *et al.* [3] evaluated the anti-inflammatory property of different selected plant species of Asteraceae to inhibition of Cyclooxygenase-1 and 5-Lipoxygenase and they find *Cichorium intybus* L. (3.6 and 0.3 $\mu\text{g}/\text{mL}$); *Minasia scapigera* H. Rob. (5.6 and 4.6 $\mu\text{g}/\text{mL}$); *Piptolepis monticola* Loeuille (6.7 and 4.8 $\mu\text{g}/\text{mL}$); *Prestelia eriopus* Sch. Bip. (6.7 and 20.6 $\mu\text{g}/\text{mL}$); *Sphagneticola trilobata* (L.) Pruskei (11.9 and 12.2 $\mu\text{g}/\text{mL}$) and *Tithonia diversifolia* (Hemsl) A. Gray (28.7 and 11.6 $\mu\text{g}/\text{mL}$) were potent towards Cyclooxygenase-1 and 5-Lipoxygenase respectively.

Table 1: Extraction yield (%) of both selected plant species in aqua-methanol and aqua-acetone (Mean±S.E.).

S. No.	Plant Species	Extraction yield (%)	
		Aqua methanol	Aqua acetone
1.	<i>Acmella uliginosa</i>	10.99±0.08 ^{bcd}	9±0.57 ^{cd}
2.	<i>Blumea sinuata</i>	15.15±0.20 ^f	23.09±0.25 ^g

**Fig 1:** Total protein content (mg/ml of fresh weight) in fresh leaves of selected plant species**Fig 2:** Superoxide dismutase (U/mg FW) in fresh leaves of selected plant species**Fig 3:** Peroxidase (nmol/mg of protein) in fresh leaves of selected plant species**Fig 4:** Catalase (μmole/mg of protein) in fresh leaves of selected plant species**Fig 5:** Protein denaturation inhibition activity (%) in both aqua-methanol and aqua-acetone in selected plant species of family Asteraceae, Au= *Acmella uliginosa* Bs= *Blumea sinuata*, AM= Aqua Methanol and AA= aqua-acetone

Conclusion

The present study reveals that both plant species displayed enzymatic antioxidant potential, results showed that *B. sinuata* exhibited higher protein, SOD, POD, and CAT activities as compare to *A. uliginosa*. Methanol and acetone extract of *A. uliginosa* and *B. sinuata* showed moderate to good anti-inflammatory potential against to egg albumin. These selected plant species are weeds for agricultural lands and also have ethno-medicinal importance in different regions of the world. *A. uliginosa* also used as a leafy vegetable by tribes. Present results showed that they are potent resource of antioxidant and anti-inflammatory, it seems to be a good resource for pharmaceutical companies for development of new remedies.

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