

Evaluation of *in vitro* Anti-inflammatory Activity, Total Phenolic and Flavonoid Content of Methanol Extract of Leaves of *Acmella paniculata*

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Abstract: *Acmella paniculata* is a folk medicinal plant of Mizoram used by the folk medicinal practitioners to recuperate their different ailments. The current experiment was designed to investigate anti-inflammatory potential of methanol extracts of leaves of *Acmella paniculata* along with total phenolic content and total flavonoids content. Evaluation of total phenolics and flavonoids content showed presence of natural antioxidant agents. Inhibition of protein determination method was performed to assess in extract possess potent antioxidant property and polyphenol compounds present may help in radical related problem diseases. *In-vitro* anti-inflammatory activity study gives anti-inflammatory property when compared with standard diclofenac sodium. It has a good capability of controlling the production of auto antigen and therefore inhibit the denaturation of proteins.

Keywords: *Acmella paniculata*, anti-inflammatory, phenolics, flavonoids

1. Introduction

Acmella paniculata (Asteraceae) is an annual plant found in an area of water courses. Flower heads are said to relieve toothache or mouth problems when chewed and the leaves are used for treatment of external skin diseases. Root decoction is used as purgatives, leaf decoction as diuretic and lithotriptic; whole plant is used for dysentery [1]. The studies of anthelmintic effects [2], antimicrobial [3,4], antibacterial [5] and some antioxidant activities[3] have been reported. The current study is an attempt to determine the *in vitro* anti-inflammatory activity, total phenolic and total flavonoid content of methanol extract of the leaf.

The purpose of inflammation is to protect the site of an injury. When inflammation occurred, the body gives response and fight the invading organisms so as to destroy them and set a stage for repairing the injured tissues [6]. This happens due to the release of chemical mediators such as kinins, prostaglandins and histamines from the injured tissues [7]. Non-steroidal anti-inflammatory drugs are mainly used for curing inflammation in the body, but adverse effects like gastric irritation can occur. Traditional medicines have played vital role due to low incidence of their adverse effects and modern medicines have developed significantly from natural products. Natural antioxidants such as phenolics and flavonoids can scavenge free radicals and helps to prevent the body from oxidative stress induced damage and are found to have therapeutic properties against diseases due to oxidative stress [8]. Natural antioxidants present in plants can be helpful in preventing free radical related diseases [9]. Consumption of plant foods and natural antioxidant supplements may protect the body against diseases like cancer, neurodegenerative and cardiovascular diseases [10]. Researchers find it important to study different traditional medicines worldwide on different plant species and their active therapeutic principles which will help in discovering a newer compound with significant activities [6].

2. Materials and Methods

2.1 Plant materials

Leaves of *Acmella paniculata* were collected from DIET Campus, Chaltlang, Aizawl, Mizoram and authenticated from the Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya (No. BSI/ERC/Tech./Plant Iden./2016/321). Samples were air dried, grinded into coarse powder.

2.2 Preparation of methanol extract

Dried powdered leaf (500g) was extracted with methanol solvent by using Soxhlet extractor at 50-60°C for 24 hours. The extract was filtered and evaporated using rotary vacuum evaporator at 40°C and the extract obtained was stored in refrigerator at 4°C for further studies.

2.3 *In-vitro* anti-inflammatory activity

This study was carried out by using inhibition of protein denaturation method [11]. Test solution consists of 0.45ml BSA 5% and 0.05ml test solution. Test control solution consists of 0.45ml BSA 5% and 0.05ml distilled water. Standard solution consists of 0.45ml of BSA 5% and 0.05ml of diclofenac sodium. The above solutions were adjusted to pH 6.3 with 1N HCl and were incubated at 37°C for 20 minutes and temperature was increased at 57°C for 3 minutes. After cooling, 2.5ml of PBS pH 7.4 were added to each solutions and absorbance was measured at 416nm. Percentage inhibition (I) of protein denaturation was calculated as,

$$I(\%) = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100$$

The control reading represents 100% protein denaturation and tests results were compared with standard drug diclofenac sodium.

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2.4 Total phenolic content:

The amount of the total phenolics was measured by using Fohlin-Ciocalteu method [12]. 1ml of the extract solution at was added to test tubes containing 5ml of Fohlin-Ciocalteu reagent. After 3 min, 4ml sodium carbonate solution (0.7M) was added to each and kept for 1h in a room temperature. Gallic acid was used as standard and absorbance was taken at 765nm. The amount of phenolic content was determined and expressed as milligrams of gallic equivalent (GAE)/g of the dried extract.

2.5 Total flavonoids content

For determination of total flavonoids content quantitatively, aluminium chloride method was used [13]. An aliquot of 1ml of the methanol extract solution was mixed with 2ml of deionized water and kept for 5 minutes. 3ml sodium nitrite solution (5%) and 0.3ml aluminium chloride solutions were added and kept for 6 minutes. Then, 2ml sodium hydroxide (1M, 10%) was added and the volume was made up to 10ml with deionized water and kept for 1h. Quercetin was used as a standard and absorbance was measured at 510nm. Results were expressed as quercetin equivalents in milligram per gram (mgQE/g) of dried extract.

3. Results and Discussion

3.1 In-vitro anti-inflammatory activity

Denaturation of proteins is a well documented cause of inflammation. The anti-inflammatory property of bovine serum albumin was mainly due to the presence of two interesting binding sites in the aromatic tyrosine and aliphatic threonine and lysine residue regions of bovine serum albumin. It has been reported that the therapeutic molecules could be activating the tyrosine receptor dually with threonine that regulates signal transduction biological pathways for their overall biological action [14]. Compounds interacting with the aliphatic region around the lysine residue on bovine serum albumin is also interesting for the antioxidant activity study. Anti-inflammatory activity of many plants are due to their flavonoids content, their high sterol and triterpene contents [15]. From the present study, it can be concluded that methanol extract of the leaf of *Acmella paniculata* is effective in inhibiting the denaturation of protein and activity increased when concentration increases. Evaluation was done in triplicate and the results were presented in table 1 and figure 1.

Table 1: Percent inhibition of In-vitro anti-inflammatory activity of *Acmella paniculata* methanol extract

Concentration ($\mu\text{g/ml}$)	Percentage inhibition (%)
Standard - Diclofenac sodium	
100	28.50
200	35.30
300	41.33
400	45.78
500	49.98
Methanol Extract	
100	11.22
200	16.20
300	24.12
400	35.56
500	46.45

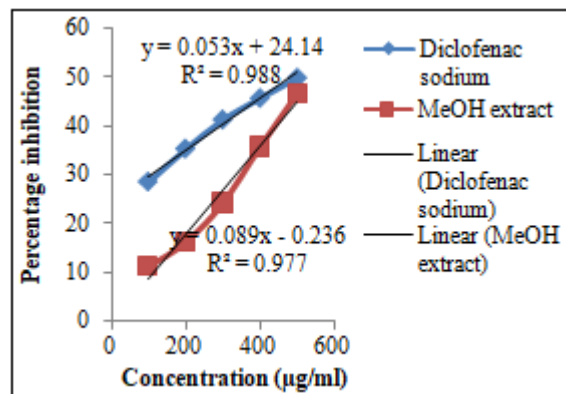


Figure 1: Percentage inhibition of diclofenac sodium and methanol extract of *Acmella paniculata* against denaturation of protein

3.2 Total phenolic and total flavonoid content

Results showed that methanol extract of *Acmella paniculata* leaf has a good phenolic content. Phenolic compounds are important plant constituents which are responsible for the antioxidant activity of plant material. They exhibit antioxidant activity by inactivating lipid free radicals or preventing the decomposition of hydroperoxides into free radicals [16,17]. Flavonoids are an important natural phenolics which possess a broad spectrum of biological and chemical activities [18]. In this study, presence of flavonoids is more than that of phenolic content in the methanol extract of leaf of *Acmella paniculata*. Results were shown in Table 3.

Table 2: Total phenolic and total flavonoid contents of methanol extract of *Acmella paniculata*.

Plant Extract	Total phenols (mg/g Gallic acid) Mean \pm SEM	Total flavonoids (mg/g Quercetin) Mean \pm SEM
Methanol extract	17.256 \pm 1.247	33 \pm 1.528

Data represented as Mean \pm SEM (n=3)

4. Conclusion

The results have clearly demonstrated that methanol extract of *Acmella paniculata* possess anti-inflammatory activity and it gives an idea that the compound present in it can be used as a lead compound for designing an anti-inflammatory drug. The extract also possesses phenolics and flavonoids, therefore the plant may be considered as a good source of natural antioxidant for medicinal purposes.

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