

In vitro anti-inflammatory and anti-ulcer activities of aqueous extract of *Emilia sonchifolia*

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Abstract

Emilia sonchifolia, an annual herb belonging to the Asteraceae family, has long been recognized for its potential in treating cutaneous infections, inflammation, and wounds. This study aimed to assess the anti-inflammatory and anti-ulcer effects of *Emilia sonchifolia* using protein denaturation assay and Acid Neutralizing Capacity (ANC) methods. The acid neutralizing capacity method revealed that the extract significantly reduced acidity to 8.35 at a concentration of 1000 mg/ml, outperforming the standard Aluminum hydroxide + Magnesium hydroxide combination which achieved a value of 13.2 at 500 mg/ml. Furthermore, the results from the protein denaturation assay demonstrated a positive correlation between increased concentration and percentage inhibition of inflammation. The extract exhibited the highest inhibition percentage of 44.99 at a concentration of 200 µg/ml, and the standard diclofenac sodium achieved 84.120 at 100 µg/ml. These findings underscore the remarkable medicinal potential of aqueous extract of *Emilia sonchifolia*, particularly in its applications as an anti-inflammatory and anti-ulcer agent. In summary, the aqueous extract of *Emilia sonchifolia* demonstrated significant anti-inflammatory activity across various concentrations, when compared to the effectiveness of the standard drug diclofenac sodium. Additionally, the extract displayed anti-ulcer activity when compared to the standard Aluminum hydroxide + Magnesium hydroxide combination. Phytochemical analysis unveiled the presence of alkaloids, glycosides, saponins, flavonoids, terpenoids, phenolic compounds, steroids, and carbohydrates in *Emilia sonchifolia*. These phytochemical constituents likely contribute to the extract's protective properties against protein denaturation and its anti-ulcer effects.

Keywords: *Emilia sonchifolia*; Anti-inflammatory; Protein denaturation; BSA; Antiulcer; Acid neutralizing capacity

1. Introduction

Plants have been extensively used to treat human diseases from the beginning of humanity. Medicinal plants produce a number of secondary metabolites that can be used for the creation of therapeutically advantageous powerful drugs. Pharmacological benefits associated with these metabolites can be proved by the assistance of evidence-based research and theories ¹.

According to World Health Organization (WHO), underdeveloped countries mainly rely on plant derived medications for treating ailments and globally more than 20,000 plants have been identified as therapeutically active. In this article a deliberative effort has been made to investigate anti-inflammatory and anti-ulcer properties of the plant '*Emilia sonchifolia*'².

Emilia sonchifolia, also known as lilac tasselflower, purple sow thistle or cupids shaving brush is an important edible medicinal plant coming under Asteraceae family. They are widely distributed throughout India as a weed in the cultivated fields, wasteland area, grassy fields, roadsides and teak forests. It is considered to be one among the "Ten

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Sacred Flowers’ of Kerala, state in India, collectively known as ‘Dasapushpam’ (Dhasha: ten, pushpam: flower). In Malayalam, it is called “Muyalcheviyan.”

It is an annual herb with a branched taproot. Stems are weak, erect or often branched at the base, smooth or sparingly hairy, 10 to 60 cm tall. This species is recognized by the Sow thistle like leaves. Lower leaves are deeply and irregularly toothed, kidney-shaped, ovate, triangular-ovate or obovate, 4-16 cm long, 1-8 cm wide with narrowly winged stalks. Upper leaves are smaller, alternately arranged, usually entire, sometimes coarsely toothed, stalkless and somewhat clasping the main stem. Inflorescence is an involucre flower head resembling a single flower, 1.2-1.4 cm long, 4-5 mm wide, urn-shaped, long-stalked, at the end of branches. Flowering branches usually dichotomously branched with 3-6 heads, each head or capitulum a composite of numerous florets. The cup of the flower-head is green, cylindrical, somewhat inflated below. Florets are 30-60 per head, purple, scarlet, red, pink, orange, white or lilac ³.

The phytochemical constituents present in *Emilia sonchifolia* are flavonoids, saponins, alkaloids, terpenes, glycosides, phenolic acids, carotenoid derivatives, etc ⁴.

1.1. Plant distribution

The native range of this species is Tropical & Subtropical old world.

Native to:

Angola, Assam, Bangladesh, Benin, Cambodia, Cameroon, China North-Central, China South-Central, China Southeast, Congo, East Himalaya, Gabon, Guinea-Bissau, Gulf of Guinea Is., Hainan, India, Ivory Coast, Japan, Jawa, Laccadive Is., Madagascar, Malaya, Mauritius, Mozambique, Myanmar, Nansei-shoto, Nepal, Nigeria, Philippines, Réunion, Senegal, Seychelles, South China Sea, Sri Lanka, Sumatera, Taiwan, Thailand, Vietnam, Yemen, Zaire.

Introduced into:

Belize, Bolivia, Brazil Southeast, Caroline Is., Cocos (Keeling) Is., Colombia, Comoros, Cook Is., Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Florida, French Guiana, Georgia, Guatemala, Guyana, Haiti, Hawaii, Honduras, Jamaica, Leeward Is., Line Is., Maldives, Marianas, Mexico Central, Mexico Southeast, New Caledonia, New South Wales, Nicaragua, Niue, Northern Territory, Panamá, Peru, Puerto Rico, Queensland, Samoa, Society Is., South Carolina, Suriname, Tonga, Trinidad-Tobago, Tuamotu, Tubuai Is., Vanuatu, Venezuela, Venezuelan Antilles, Western Australia, Windward Is ^{5,6}.

1.2. Scientific classification of *Emilia sonchifolia* ⁷

Table 1 Scientific classification of *Emilia sonchifolia*

Kingdom	<i>Plantae</i> - Plants
Sub kingdom	<i>Tracheobionta</i> - Vascular plants
Super division	<i>Spermatophyta</i> - Seed plants
Division	<i>Magnoliophyta</i> - Flowering plants
Class	<i>Magnoliopsida</i> - Dicotyledons
Subclass	<i>Asteridae</i>
Order	<i>Asterales</i>
Family	<i>Asteraceae</i>
Genus	<i>Emilia</i>
Species	<i>Emilia sonchifolia</i>

1.3. Uses

- The leaves juice of *Emilia sonchifolia* is used for the treatment of eye inflammations, night blindness, cuts and wounds, sore ears, infantile tympanites and bowel complaints.
- The leaves are also used against fever and dysentery.

- The flower heads are chewed and kept in the mouth for about 10 minutes to prevent tooth decay
- This plant has astringent, depurative, diuretic, expectorant, febrifuge and sudorific properties.
- Diverse biological activities like cytotoxic, antitumor, anti-inflammatory, analgesic, antinociceptive, antiulcer, modulatory effects, antiviral, erythropoietic and hepatoprotective of the *Emilia sonchifolia* have been reported^{8,9}.

2. Material and methods

2.1. Collection and authentication of the plant

Whole plant of *Emilia sonchifolia* Linn. (DC.) were collected in April 2023 from Peechi forest area, Thrissur district, Kerala, India. The plant was authenticated by Dr. Madiga Beemalingappa, Scientist B, Forest Botany Department, Kerala Forest Research Institute (KFRI), Peechi, where a voucher specimen is maintained.



Figure 1 *Emilia sonchifolia*

2.2. Preparation of extract

The whole plant was washed thoroughly with sufficient water and chopped into smaller pieces. It was dried under shade for 3 weeks and reduced to coarse powder using an electrical blender and kept in an airtight container with proper labelling for future use. The powdered plant material (72.5 g) was extracted by macerating in distilled water (500 ml) for 72 hours. The liquid extract was strained using muslin cloth, allowed to cool and filtered. The filtrate was concentrated using heating mantle at 35 °C. The extract was stored in refrigerator at 4 °C until used for the experiment¹⁰.

2.3. Qualitative Chemical Evaluation

2.3.1. Phytochemical screening

The aqueous plant extract of the whole plant of *Emilia sonchifolia* were subjected to chemical tests to identify the constituents present in the plant.

2.3.2. Detection of alkaloids

The extract was mixed with few drops of dil. HCl and was then filtered. Test for alkaloids was carried out in this filtrate.

Mayer's Test

One or two drops of Mayer's reagent (mercuric chloride 1.36 g dissolved in 60ml distilled water and mixed in a solution of 5 g of potassium iodide in 10 ml distilled water) was added to the filtrate through the sides of the test tube, formation of white creamy precipitate indicates the presence of alkaloids.

Wagner's Test

Wagner's reagent (1.27 g of iodine and 2 g potassium iodide dissolved in 5 ml distilled H₂O) was added to the filtrate. Formation of reddish-brown precipitate indicates the presence of alkaloids.

2.3.3. Detection of glycosides

Keller killiani test

To 2 ml of extract, added glacial acetic acid, 1 drop of 5% FeCl₃ and conc. H₂SO₄. Reddish brown color appears at the junction of the 2 liquid layers and upper layer appears bluish green.

Baljet's test

To a few ml of extract, added 1 ml of sodium picrate solution and observed for the color change from yellow to orange which reveals the presence of glycosides.

2.3.4. Detection of terpenoids

Salkowski test

5 ml of extract was mixed with 2 ml of chloroform and about 3 ml of conc.H₂SO₄ was carefully added. At the separation level of the two liquids, a reddish-brown ring forms, this indicates the presence of terpenoids.

2.3.5. Detection of carotenoids

About 0.02 g of plant extract was mixed with chloroform, mixed well and then the mixture was filtered. To the filtrate, conc. H₂SO₄ was added, formation of a blue color at the interface indicates the presence of carotenoids.

2.3.6. Detection of steroids

Libermann-Burchard test

1 ml of extract was treated with 0.5 ml of acetic anhydride and 1 ml of H₂SO₄ carefully. A color change from violet to blue or green indicates the presence of steroids.

2.3.7. Detection of saponin

Foam test

About 0.5 gm of extract was mixed with 2 ml of distilled water and heated for few minutes and filtered. The filtrate was vigorously shaken. The persistent froth was observed for 10 minutes, this indicates the presence of saponins.

2.3.8. Detection of flavonoids

- The extract was shaken with 1 ml of dilute ammonia solution and conc.H₂SO₄. Formation of yellow color indicates the presence of flavonoids.
- **Aqueous sodium hydroxide test**
To a few ml of extract, aqueous NaOH was added. Formation of yellowish orange color indicates the presence of flavonoids.

2.3.9. Detection of phenolics

Ferric chloride test

To the plant extract, a few drops of 1% aqueous or alcoholic ferric chloride were added. The formation of bluish-black color indicates the presence of phenol.

Lead acetate test

A small quantity of extract was dissolved in distilled water and 3 ml of 10% lead acetate solution was added to it. A bulky white precipitate indicates the presence of phenolic compounds.

2.3.10. Detection of quinine

One ml of the plant extract was mixed with 5 ml of con. HCl. The formation of yellow precipitate indicates the presence of quinone.

2.3.11. Detection of tannin

The sample was mixed with distilled water and boiled for 5 minutes. It was filtered and was used for the test. Two drops of 10% ferric chloride were added to 1 ml of the filtrate. Formation of bluish or greenish or brownish black color indicates the presence of tannins.

2.3.12. Detection of carbohydrates

Molisch test

2 ml of the prepared filtrate were mixed with 0.2 ml of alcoholic solution of α -naphthol 10% and 2 ml of sulphuric acid, a reddish violet zone is formed, this indicates the presence of carbohydrates or glycosides^{11,12,13}.

2.4. In vitro anti-inflammatory activity

2.4.1. Protein denaturation method using Bovine Serum Albumin (BSA)

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation¹⁴.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed medications in the world because of their verified effectiveness in reducing pain and inflammation. NSAIDs has accounted for prevention of the protein denaturation, which acts as antigens and prompts autoimmune diseases. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation¹⁵.

- Preparation of 0.5% Bovine Serum Albumin (BSA)

Dissolved 500 mg of BSA in 100 ml of water¹⁶.

- Preparation of Phosphate buffer solution (pH 6.4)

Dissolve 2.5 g of disodium hydrogen phosphate, 2.5 g of sodium dihydrogen phosphate and 8.2 g of sodium chloride in 950 ml of water. Adjust the pH of the solution to 6.4 with 1 M sodium hydroxide or 1 M hydrochloric acid, if necessary. Dilute to 1000.0 ml with water.

- Preparation of test solution

Test solution (0.5 ml) consists of 0.45 ml of Bovine serum albumin (0.5% w/v aqueous solution) and 0.05 ml of test solution of various concentrations.

- Preparation of standard Diclofenac

Standard solution (0.5 ml) consists of 0.45 ml of Bovine serum albumin (0.5% w/v aqueous solution) and 0.05 ml of Diclofenac sodium of various concentrations¹⁷.

- Procedure

0.05 ml various concentrations (6.25, 12.5, 25, 50, 100, 200 μ g/ml) of test formulations and standard drug diclofenac sodium (6.25, 12.5, 25, 50, 100 μ g/ml) were taken, respectively. Then 0.45 ml (0.5% w/v) BSA mixed in all tubes. The samples were incubated at 37 °C for 20 minutes and the temperature was increased to keep the samples at 57 °C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 660 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium.

The percentage inhibition of protein denaturation can be calculated by

$$\text{percentage inhibition} = \left[\frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}} \right] \times 100$$

Were,

Abs test = Absorbance of the test sample

Abs control = Absorbance of control ^{14,18,19}.

2.5. *In vitro* Antiulcer activity

2.5.1. Acid Neutralizing Capacity (ANC) importance

The acid-neutralizing capacity (ANC) is the amount of acid that can be neutralized by an antacid. The United States Pharmacopoeia (USP) describes the ANC test as a back-titration method using sodium hydroxide (0.5 N solution) to a set endpoint of pH 3.5 to determine the number of milliequivalents of acid (hydrochloric acid 1 N solution) neutralized by the minimum labeled dosage (MLD) of an antacid ²⁰.

Antacids are alkaline drugs that neutralize gastric acidity and exert a buffering effect to stabilize the pH of the gastric juice. They are divided into 2 categories: absorbable compounds such as sodium bicarbonate, calcium carbonate (CaCO₃), and magnesium carbonate (MgCO₃), and non-absorbable compounds such as aluminum phosphate (AlPO₄), aluminum hydroxide (Al (OH)₃), and magnesium hydroxide (Mg (OH)₂). They are usually marketed as a combination of 2 or 3 compounds ²¹.

2.5.2. Acid Neutralizing Capacity (ANC) Method

The acid neutralizing capacity (ANC) value for aqueous extract of whole plant of *Emilia sonchifolia* in different concentrations (100 mg/ml, 200 mg/ml, 500 mg/ml, 1000 mg/ml) were compared with the standard antacid AHMH (aluminum hydroxide + magnesium hydroxide -500 mg/ml) ²².

To the 5 ml quantity of each extract individually, water was added and mixed well to make up the total volume up to 70 ml. Then 30 ml of 1 N HCl was added into standard and test preparation and stirred for 15 minutes, 2-3 drops of phenolphthalein solution was added and mixed. The excess HCl was immediately titrated with 0.5 N Sodium hydroxide solution drop wise until a pink color is appeared ²³.

The moles of acid neutralized is calculated by,

$$\text{Total mEq of acid consumed} = (\text{vol. of HCl} \times \text{Normality of HCl}) - (\text{vol. of NaOH} \times \text{Normality of NaOH})$$

Acid neutralizing capacity (ANC)

$$\text{per gram of antacid} = \frac{\text{Moles of HCl neutralized}}{\text{Grams of Antacid or Extract}}$$

Calculating the moles of acid neutralized involves dividing the volume of HCl by its normality and the volume of NaOH by its normality. Moles of HCl neutralized divided by grammes of antacid/extract equals acid neutralizing capability (ANC) per gramme of antacid ^{24,25,26}.

3. Results and discussion

The whole plant of *Emilia sonchifolia* was dried, powdered and extracted using water as solvent by cold maceration method. The percentage yield of aqueous extract of *Emilia sonchifolia* were calculated and depicted in the table 2.

Table 2 Percentage yield of aqueous plant extract of *Emilia sonchifolia*

Extract	Colour & Consistency	Yield (g)	% yield
Aqueous extract	Dark green & semisolid	20.65	28.48 %w/v

3.1. Phytochemical Screening

Table 3 Detection of compounds

Sl. No.	Chemical test	Aqueous extract
1.	Detection of alkaloids	
	Mayer's test	+
	Wagner's test	+
2.	Detection of glycosides	
	Keller-Killiani test	+
	Baljet's test	-
3.	Detection of terpenoids	
	Salkowski test	+
4.	Detection of carotenoids	
		-
5.	Detection of steroids	
	Libermann- Burchard test	+
6.	Detection of saponins	
	Foam test	+
7.	Detection of flavonoids	
	Aqueous sodium hydroxide test	+
8.	Detection of phenolics	
	Ferric chloride test	+
	Lead acetate test	+
9.	Detection of quinine	
		-
10.	Detection of tannin	
		+
11.	Detection of carbohydrates	
	Molisch test	+

From the above chemical tests, the plant shows the presence of alkaloids, glycosides, phenolics, terpenoids, steroids, flavonoids, carbohydrates and saponins.

3.2. *In vitro* anti-inflammatory activity

3.2.1. Protein denaturation

Anti-inflammatory activity of *Emilia sonchifolia* by protein denaturation using Bovine Serum Albumin (BSA).

The *In vitro* anti-inflammatory activity of aqueous extract of *Emilia sonchifolia* at different concentration was performed by Protein denaturation assay and the results were shown in table 4. From the results it was observed that the percentage inhibition of inflammation increased with increased concentration. The higher percentage of inhibition was obtained at the concentration of 200 ($\mu\text{g/ml}$) found to be 44.99, it was comparable with diclofenac sodium standard 84.12 at 100 ($\mu\text{g/ml}$)^{27,28}.

Table 4 Denaturation of BSA* by Diclofenac Sodium and AEES*

Sample	Concentration (µg/ml)	Absorbance(nm)	% of inhibition
Diclofenac Sodium (Standard)	Control	0.787	-
	6.25	0.725	7.88
	12.5	0.661	16.01
	25	0.545	30.75
	50	0.368	53.24
	100	0.125	84.12
AEES (Test)	Control	0.898	-
	6.25	0.855	4.79
	12.5	0.788	12.25
	25	0.724	19.38
	50	0.659	26.61
	100	0.599	33.30
	200	0.494	44.99

*BSA: Bovine Serum Albumin; *AEES: Aqueous Extract of *Emilia sonchifolia*

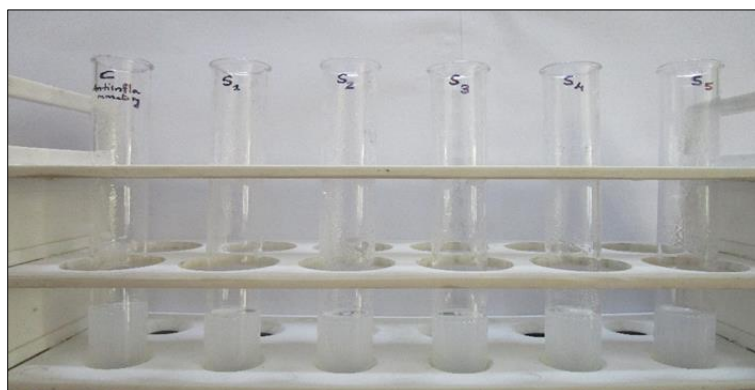


Figure 2 Test tubes containing standard diclofenac sodium

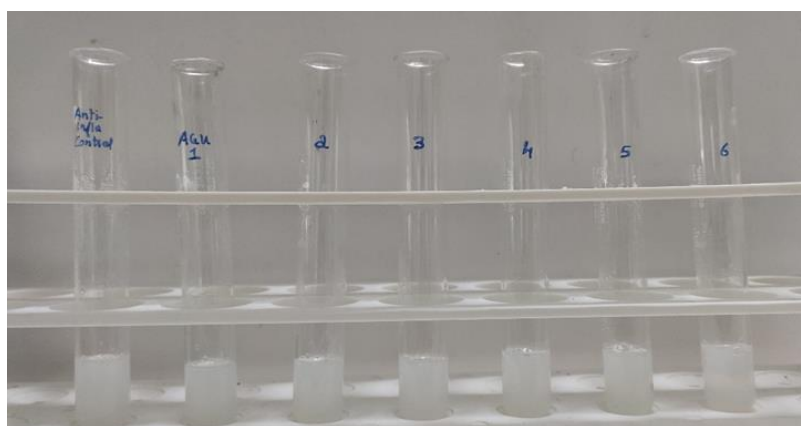


Figure 3 Test tubes containing test sample AEES

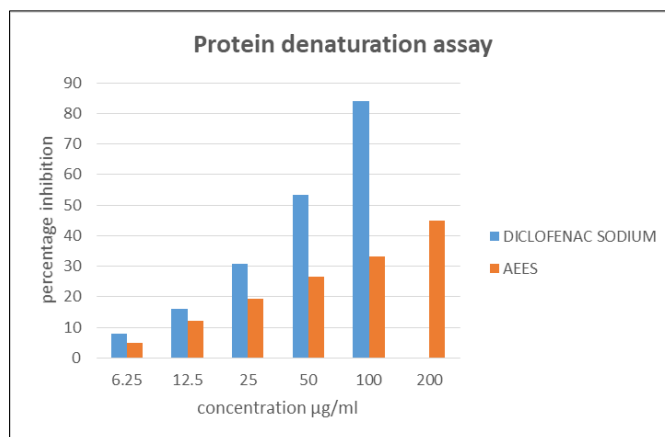


Figure 4 Percentage denaturation of BSA by Diclofenac sodium and AEES

3.3. *In-vitro* anti - ulcer activity

The *in-vitro* acid neutralizing effects of aqueous extract of whole plant of *Emilia sonchifolia* in different concentrations (100 mg, 200 mg, 500 mg, and 1000 mg per ml) were compared with the standard antacid AHMH-500 mg/ml. The results showed concentration dependent reduction in acid neutralizing capacity per gram of antacid was found as 158.5, 62, 22.5 and 8.35 respectively. Similarly, ANC value of the standard AHMH (500 mg) was found to be 13.2 which is quite similar to the concentration of test drug. Whereas, test drug concentration 1000 mg was found to neutralize acid more significantly as compared to standard^{29,30}. The results are tabulated in Table 5.

Table 5 Acid Neutralizing Capacity (ANC) of aqueous extract of whole plant of *Emilia sonchifolia* by In vitro method

Concentration (mg/ml)	Volume of NaOH consumed (ml)	mEq of acid consumed	ANC per gram of antacid
AEES*: 100 mg	28.3	15.85	158.5
AEES: 200 mg	35.2	12.4	62
AEES: 500 mg	37.5	11.25	22.5
AEES: 1000 mg	43.8	8.35	8.35
AHMH*: 500 mg	46.8	6.6	13.2

*AEES: Aqueous Extract of *Emilia sonchifolia*; *AHMH: Aluminium Hydroxide + Magnesium Hydroxide

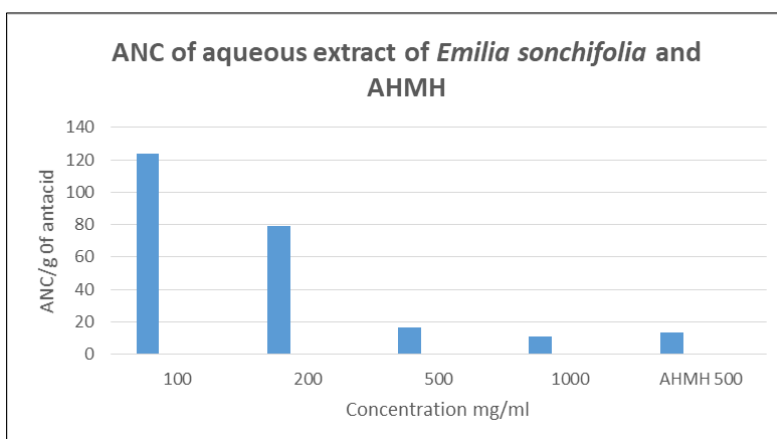


Figure 5 *In vitro* acid neutralizing capacity of aqueous extract of *Emilia sonchifolia* and AHMH

4. Conclusion

Emilia sonchifolia commonly known as 'lilac tassel flower' or 'muyalcheviyan' in Malayalam is an annual herb of the family 'Asteraceae'. Due to its several ethnomedical uses, it has been investigated for a good number of pharmacological activities. The results of this study revealed that aqueous extracts of *Emilia sonchifolia* possess anti-inflammatory and anti-ulcer properties. These properties may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, steroids and phenols.

The extract fractions serve as free radical inhibitors or scavengers or acting possibly as primary oxidants and inhibited the protein denaturation. The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e., inhibition of protein denaturation by *Emilia sonchifolia* extract and reference drug diclofenac sodium. It is a scientific validation of its traditional uses in the management of inflammation.

The acid neutralizing property of the plant was determined by back titration method which was found useful in neutralizing the HCl. Results from ANC indicated a concentration-dependent decrease in acid neutralizing capacity compared to each gram of antacid administered.

Therefore, this study gives an idea that the aqueous extracts of the plant *Emilia sonchifolia* can be used as lead compound for designing a potent anti-inflammatory or anti-ulcer drug. However further definitive studies are required via detailed experimentation to ascertain the mechanisms and constituents for its properties.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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