



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203





Human Journals

Research Article

July 2023 Vol.:27, Issue:4

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## In Vitro Investigation of Anti Inflammatory Activity of Leaves Extract of *Tradescantia Spathacea* Swartz.

	
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<b>Submitted:</b> 30 June 2023	
<b>Accepted:</b> 20 July 2023	
<b>Published:</b> 30 July 2023	

**Keywords:** *Tradescantia Spathacea* (Oyster Plant), Leaves Extract, Anti-Inflammatory Activity, Egg Albumin Denaturation Assay, Trypsinase Inhibition

### ABSTRACT

Medicinal plants are for the discovery and development of new therapeutic agents with increased efficiency to solve various problems related to human health. *Tradescantia spathacea* sw plant is used to treat various diseased conditions. *Tradescantia spathacea* Swartz is plant belong to the family Commelinaceae. Leaves of *Tradescantia spathacea* show various activity like Anti-Helminthic, Antimicrobial, and Anti-Coagulant activity but there no study reported study on the in vitro anti-inflammatory activity of the leaf extract of *Tradescantia spathacea*. In the present study the in vitro anti-inflammatory activity of methanol and Aqueous extract of *Tradescantia spathacea* (Swartz) leaves were evaluated. The activity is evaluated using the in vitro protein denaturation bioassay and trypsinase inhibitory activity. The denaturation of protein is induced by heat. The anti-inflammatory activity is found to be significant in methanol extract compared to the aqueous extract. Methanol extract shows 74.15% of inhibition to protein denaturation at 500µg/ml concentration compared to the standard Aspirin and shows 77.52% of inhibition to trypsinase at 500µg/ml concentration compared to the standard Aspirin.



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## INTRODUCTION

Natural products for the treatment and management of severe illnesses may be found in medicinal plants. The use of plant extracts and isolated pure compounds has provided the basis for the production of herbal medicines and phytopharmaceutical compounds.[1] medicinal plants are for the discovery and development of new therapeutic agents with increased efficiency to solve various problems related to human health.[2] An essential non-specific defense response to tissue injury, such as that brought on by a virus or wound, is inflammation, which is characterized by warmth, redness, pain, and swelling. Inflammation can occur when viruses or infectious microorganisms such as bacteria and fungi, invade the body, also in response to tissue injuries, cell death, cancer, ischemia, and degeneration.[3] Innate immune and adaptive immune responses are involved in the formation of inflammation. Both are defense mechanisms against invasive pathogens and cancerous cells. The use of plants, their parts, and extracts as an anti-inflammatory is widespread in several geographical areas.[4] *Tradescantia spathacea*, or Moses-in-the-Cradle, is an herb native to Mexico with fleshy rhizomes. It has rosettes of waxy lance-shaped leaves. Leaves are dark to metallic green above, with glossy purple underneath. These will reach up to 1 foot (30 cm) long by 3 inches (7.5 cm) wide. They are very attractive foliage plants that will reach 1 foot (30 cm) tall. They are hardy in USDA zones 9-12. [5] It is invasive and exotic to South Florida. The current research was based on the In-vitro Anti-inflammatory activity in *Tradescantia spathacea*. This was the first report for the plant and no systematic work has been undergone in this plant.[6] Despite these traditional applications, *R. spathacea* remains uncommonly used outside of South America, but shows promise to be established as a beverage internationally.[7]

In Asia *Tradescantia spathacea* are used to treat inflammatory conditions caused by blows, fractures, wounds and other painful traumas. Recent studies show that *Tradescantia spathacea* Swartz contains sterols, phenols, flavonoids, coumarins, alkaloids, triterpenes, mucilage and saponins. Some of these metabolites could be related to the anti-inflammatory activity describable to this plant.[8] The aim of this study was to determine the in vitro anti-inflammatory activity of the aqueous and methanol extracts of the leaves of *Tradescantia spathacea* (SW) with the help of using in vitro protein denaturation inhibition bioassay and In vitro trypsinase inhibition bioassay.[9]

## 2. MATERIALS AND METHODS

### 2.1 collection of Plant material

The fresh leaves of *Tradescantia spathacea* (SW) were collected from the collage botanical garden Methawade, Sangola, Solapur (district), Maharashtra, India in December 2022. the authenticated by Dr. Thete S.V. Dept. of Botany, KBP collage of Pandharpur, with help of flora of Solapur District, Maharashtra, India.

### 2.2 Extraction of the plant material

The leaves of *tradescantia spathacea* (SW) were shade dried at room temperature for 3 weeks. The dried parts were later coarsely powdered with the help of an electric grinder after passing through sieve no 20 to obtain coarse powder. The coarse powders (10g) were subjected to successive extraction in 250 ml of solvent (methanol and water) by using the Soxhlet apparatus. The cycles were carried out until a clear solvent was obtained (6 cycles). The collected extracts were stored and then taken up for further investigations.[10]

### 2.3 Drugs and chemicals

All other chemicals used were of analytical grade obtained commercially. Egg albumin, disodium hydrogen phosphate, potassium dihydrogen phosphate, Trypsin, Tris HCL buffer, casein, Sodium Chloride, tri- choro acetic acid, methanol, standard Aspirin.

### 2.4 In vitro Anti-inflammatory activity

#### 2.4.1. In vitro Albumin denaturation inhibition bioassay [15][16][17]

The method for the assay of inhibition of albumin denaturation is described below for the present study. In this assay 0.9 ml of fresh egg albumin (3% aqueous solution) and 0.1 ml of test solution of *Tradescantia spathacea* sw. leaves extract of different concentrations (200 ug /ml. 300 Hg ml, 500 ug/ml prepared in methanol) was taken in different test tubes. For control 0.1 ml distilled water was used instead of the test solution. And for standard 0.1 ml of Aspirin was used instead of the test solution. (Different concentration was made in methanol viz.200 Hg /ml, 300 Hg/ ml 500 Hg/ ml). The mixture was incubated at 37°C for 5 min. Then test tubes were heated at 55°C for 3 minutes and then cool. After cooling the test tubes.2.5 ml of phosphate buffer saline pH 6.3 was added to each test tube. The absorbance was measured

at 660 nm spectrophotometrically.<sup>3</sup> The inhibition to the protein denaturation was measured as Percentage inhibition = (Abs control – Abs sample) /Abs control × 100.

### 2.4.2 In vitro trypsinized inhibition bioassay [15][16][17]

The in-vitro trypsinase inhibitory activity is carried out, in this bioassay the reaction mixture contained ml (0.06mg/ml) trypsin, 1 ml Tris-HCl buffer of pH 7.4 and 1 ml of test solution of different concentrations (Different concentration was prepared in methanol viz. 200 ug/ml, 300 ug/ ml., 500 ug/ml) were incubated at 37°C for 5 minutes. For control 1 ml of buffer solution instead of test solution was used and for standard solution 1 ml of aspirin solution (200 µg/ml, 300 µg/ ml, 500 ug/ml concentrations in methanol) was used. After incubation 1ml of 2% w/v casein was added and this whole mixture was incubated for 20 minutes at 37°C. After that 2ml of 5% Tri-choro acetic acid was added to stop the reaction. This cloudy suspension was centrifuged for 5 minutes and the absorbance of supernatant was taken at 280 nm. Buffer is used as blank. the inhibition of the protein denaturation was measured as Percentage inhibition = (Abs control – Abs sample) /Abs control × 100.

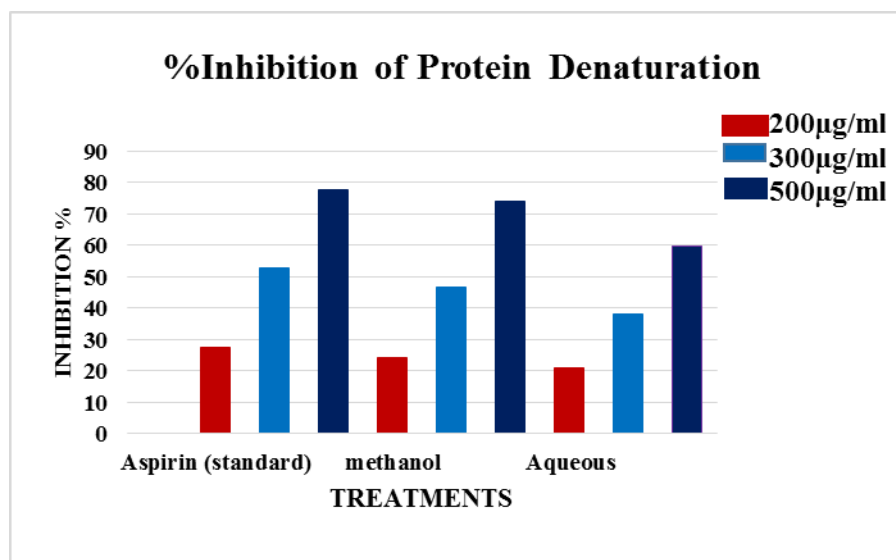
## 3. RESULT

### 3.1 In vitro protein denaturation inhibition bioassay

In vitro percentage (%) inhibition of the protein denaturation induced by the heat of methanol and aqueous extraction of *tradescantia spathacea* Swartz at different concentrations are shown in Table no 1. Percentage inhibition of both leaves extract of *Tradescantia spathacea* is compared with percentage inhibition of standard Aspirin fig no 1.

**Table 1. Protein denaturation inhibition of both extracts of *tradescantia spathacea* SW.**

Sr.no	Treatments	Concentration (µg/ml)	Absorbance (660 nm)	Inhibition %
	Control	-	0.178	-
1	Aspirin (standard)	200	0.129	27.52
		300	0.084	52.80
		500	0.040	77.52
2	Methanol Extract	200	0.135	24.15
		300	0.095	46.62
		500	0.046	74.15
3	Aqueous Extract	200	0.139	21.16
		300	0.110	38.20
		500	0.072	59.55



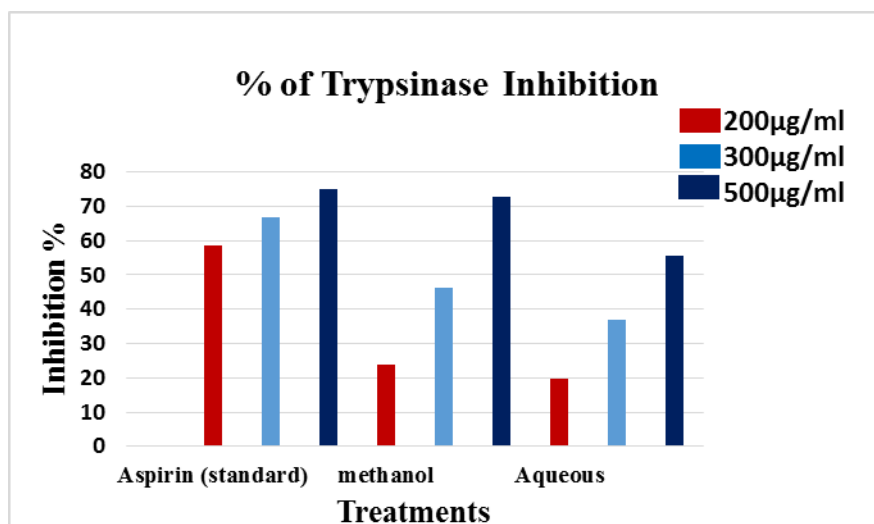
**Fig.1: Percentage inhibition of albumin denaturation inhibition of both extracts of *Tradescantia spathacea sw.***

### 3.2 In vitro trypsinized inhibition bioassay

In vitro trypsinase inhibition of both leaves extract of *tradescantia spathacea sw.* at both concentrations are shown in Table no 2. Percentage inhibition of both leaves extract of *tradescantia spathacea* is compared with percentage inhibition of standard Aspirin fig 2.

**Table 2: In vitro trypsinase inhibition of both leaves extract concentration of *tradescantia spathacea sw.***

Sr.no	Treatments	Concentrati on (µg/ml)	Absorbance (660 nm)	Inhibition %
	Control	-	0.405	-
1	Aspirin (standard)	200	0.168	58.51
		300	0.135	66.66
		500	0.101	75.06
2	Methanol Extract	200	0.309	23.70
		300	0.218	46.17
		500	0.110	72.83
3	Aqueous Extract	200	0.325	19.75
		300	0.256	36.79
		500	0.180	55.55



**Fig.2: Percentage inhibition of trypsinase at both leaves extract of *Tradescantia spathacea* sw.**

#### 4. DISCUSSION

By applying an external stressor or substance, such as a potent acid or base, a concentrated inorganic salt, an organic solvent, or heat, proteins can become denatured, losing both their secondary and tertiary structures. When denatured, the majority of biological proteins cease to function biologically. Denaturation of proteins is a well-documented cause of inflammation. 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 denaturation. Maximum inhibition of 74% was observed at 500 µg/ml. Aspirin, a standard anti-inflammation drug showed a maximum inhibition 77% at the concentration of 500 µg/ml compared with control (Table 1).

Trypsin is a proteolytic enzyme that plays a crucial role in the digestion of proteins by breaking peptide bonds. It is found in various tissues and organs, including the pancreas, and is involved in many physiological processes. However, excessive trypsin activity can be harmful and is associated with several pathological conditions, such as pancreatitis and inflammatory disorders. In vitro trypsinase inhibition bioassay exhibited significant antiproteinase activity at different concentrations as shown in Table 2. It showed maximum inhibition in methanol extract 72% at 500µg/ml. Aspirin showed maximum inhibition 75% at 500µg/ml.

## 5. CONCLUSION

From the present study of *in vitro* anti-inflammatory activity, it concludes that both leaves extract of *Tradescantia spathacea* shows potential for inhibiting inflammatory reaction. The present investigation compared the standard aspirin with extracts by simply comparing it concluded that methanol extract shows significant inhibition activity of protein denaturation and trypsinase inhibitory activity.

## 6. ACKNOWLEDGMENT

The authors are grateful to the college for providing generous support and lab facilities.

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