Asian Journal of Innovative Research. (2021): 6(1) 11-16

ISSN 2455 7285

ASIAN JOURNAL OF INNOVATIVE RESEARCH

Available online at http://www.asianjir.com

Research Article Botany

PHYTOCHEMICAL SCREENING AND ASSESSMENT OF IN VITRO ANTI-INFLAMMATORY ACTIVITY OF *Aerva lanata* L. LEAVES

M. SIVANANDHINI, Dr. S. BEATRICE FLORENCE FEBRONIA,

P.G. and Research department of botany, Kunthavai Naacchiyaar Government Arts, College for Women (Autonomous), (Affiliated to Bharathidasan University)
Thanjavur -613 007, Tamil Nadu, India

ABSTRACT

In the present study was to investigate the phytochemical screening, histochemical and anti-inflammatory activity of *Aerva lanata* leaves extract. The following conclusion observed from the study. The phytochemical screening *Aerva lanata* leaves showed that the presence of tannin, saponins, flavonoids, steroids, polyphenol, anthroquinones, glycosides, terpenoids, triterpenoids, alkaloids and coumarins in methanol and aqueous extract. Quantitative analysis showed that significant amount of phytochemicals such as flavonoids, phenol and terpenoids were present in *Aerva lanata* leaves. Histochemical studies further proved the presence of phytochemicals in *Aerva lanata* leaves. The alcoholic extract confirm the anti-inflammatory activity (Egg albumin activity of highly in 87.75 for 500µg/ml, and Bovine Serum albumin activity of highly in 86.63 for 500µg/ml)..

Keywords: *Aerva lanata*, Phytochemical, Histochemical, Anti-Inflammatory activity

INTRODUCTION

Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes /macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors vascular permeability, neutrophil chemotaxis, stimulate smooth muscle contraction have direct enzymatic activity, induce pain or mediate oxidative damage. However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhea, rheumatoid arthritis and atherosclerosis (Henson and Murphy, 1989).

A number of natural products are used in the traditional medical systems in many countries. Alternative medicine for treatment of various diseases is getting more popular. Making medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. The majority of clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutic for treatment of various inflammatory diseases. In order to investigate phytochemicals and in vitro anti-inflammatory activity of the *Aerva lanata* leaves.

MATERIALS AND METHODS

Collection of plant materials

The *Aerva lanata* leaves were collected in January 2021 from Pudukkottai, Thiruvarur district, Tamil Nadu, India. The collected *Aerva lanata* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Then examined carefully, old infected and fungus damaged portion of the leaves were removed. Healthy leaves was dried in room temperature and grind using grinder mixture. The powder was stored for further analysis.

Preparation of plant powder

1 gram of the powder of *Aerva lanata* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *Aerva lanata* leaves were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984). Total phenols estimated by the method of Edeoga et al., (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956). Histochemical tests (John Peter Paul, 2014; Gersbach et al., 2001).

***In vitro* anti-inflammatory activity**

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra et al. (2012).

RESULTS AND DISCUSSION

Qualitative and quantitative analysis

In the present study was carried out on the Aerva lanata leaves revealed the presence of medicinally active constituents. The phytochemical characters of the Aerva lanata leaves investigated and summarized in Table-1 and figure 3 and 4. The phytochemical screening Aerva lanata leaves showed that the presence of tannin, saponins, flavonoids, steroids, polyphenol, anthroquinones, glycosides, terpenoids, triterpenoids, alkaloids and coumarins in methanol and aqueous extract,

Hassain et al. (2011) screened phytochemical constituents from methanol leaf extract of Bombax malabaricum. Various organic 11 solvent extracts of Pedalium murex were subjected to preliminary phytochemical screenings by Thamizh mozhi et al. (2011). Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents. Pascaline et al. (2011) screened phytochemical constituents of some medicinal plants used by the Nandis of South Nandi District, Kenya.

Table.1: Qualitative analysis of Phytochemicals in *Aerva lanata* leaves extract

S. No	Phytochemicals	Methanol extract	Aqueous extract
1	Tannin	++	++
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	++	++
5	Terpenoids	++	++
6	Triterpeniods	++	++
7	Alkaloids	+	+
8	Antroquinone	++	++
9	Polyphenol	++	++
10	Glycosides	++	++
11	Coumarins	++	++

(+) Presence, (++) High concentrations and (–) Absences

Quantitative analysis

Quantitative analysis revealed that the *Aerva lanata* leaves powder has significant amount of flavonoids (20.00mg/gm), polyphenol (167.00mg/gm) and Terpenoids (10.00mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

Table.2: Quantitative analysis of Phytochemicals in *Aerva lanata* leaves powder

Phytochemicals	Results (mg/gm)
Poly phenol	167.00 ± 11.69
Flavonoids	20.00 ± 1.40
Terpenoids	10.00 ± 0.70

Values are expressed as Mean ± SD for triplicates

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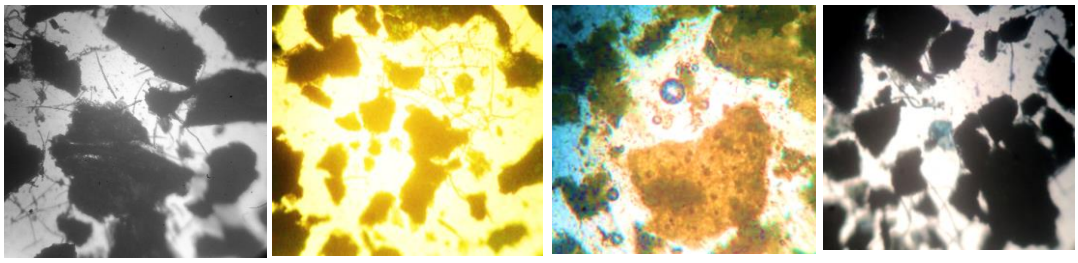
Histochemical analysis of *Aerva lanata* leaves powder

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues; it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytocompounds (Krishnan *et al.*, 2001). In the present study, *Aerva lanata* leaves powder were treated with specific chemicals and reagents. The *Aerva lanata* leaves powder treated with diluted ammonia and H_2SO_4 gave yellow colour lanatates Flavonoids, treated with $FeCL_3$ reagent gave dark blue to black colour lanatates Tannin, treated with Few drops toluidine blue reagent gave Blue green / red colour lanatates Polyphenol. (Table 3**)**. This results further confirmed the presence of phytochemicals.

Table.3: Histochemical analysis of *Aerva lanata* leaves powder

S. No	Phytochemicals	Results
1	Tannin	+
2	Flavonoids	+
3	Terpenoids	++
4	Polyphenol	+

Note: (+) Presence; (++) present with high intensity of the colour



Tannin **Flavonoids** **Terpenoids** **Polyphenol**

Fig.4: Histochemical analysis of *Aerva lanata* leaves powder

***In vitro* Anti-inflammatory activity of *Aerva lanata* leaves extract**

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property *Aerva lanata* leaves. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in arthritic diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Grant et al., 1970). Agents that can prevent protein denaturation therefore, would be worthwhile for anti- arthritic drug development. The increments in absorbance of test samples with respect to control lanatated stabilization of protein (Membrane stabilizing activity & bovine albumin) denaturation by and reference diclofenac sodium. *Aerva lanata* leaves exhibited anti-inflammatory activities in dose dependent manner.

Present study was alcoholic *Aerva lanata* leaves extract confirm the anti-inflammatory activity in Egg albumin activity of highly in 87.75 for 500µg/ml, and Bovine Serum albumin activity of highly in 86.63 for 500µg/ml (Table 4 and 5, fig 5,6).

Table.4: *In vitro* anti- inflammatory activity of *Aerva lanata* leaves (Egg albumin)

Concentrations (µg/ml)	*Aerva lanata* leaves	Standard (Diclofenac sodium)
100	13.20 ± 0.66	20.18 ± 0.87
200	48.29 ± 0.56	50.54 ± 1.03
300	60.13 ± 0.58	67.15 ± 0.67
400	76.71 ± 0.44	79.32 ± 0.82
500	87.75 ± 0.64	91.74± 1.07
IC_{50}	259.38	231.43

Values are expressed as Mean ± SD for triplicates

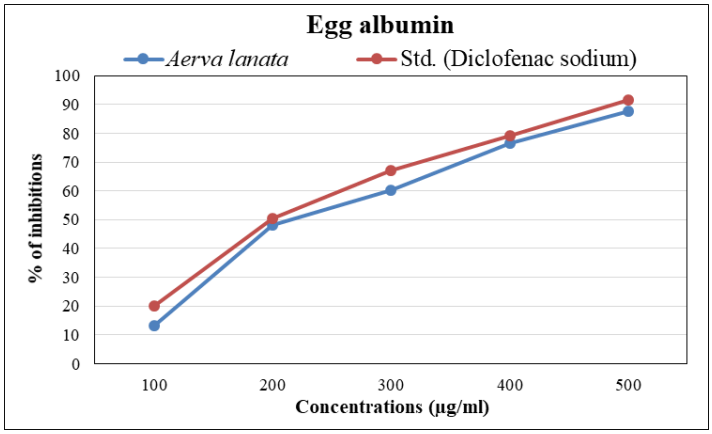


Fig.5: *In vitro* anti- inflammatory activity of *Aerva lanata* leaves (Egg albumin)

Table.5: *In vitro* anti-inflammatory activity of *Aerva lanata* leaves (Bovine Serum albumin)

Concentrations (µg/ml)	*Aerva lanata* leaves	Standard (Diclofenac sodium)
100	12.39 ± 1.45	19.32 ± 1.28
200	32.68 ± 3.62	41.86 ± 3.39
300	62.63 ± 2.73	67.72 ± 2.41
400	75.52 ± 3.75	82.45 ± 2.67
500	86.63 ± 4.02	93.68 ± 3.97
IC_{50}	279.27	241.87

Values are expressed as Mean ± SD for triplicates

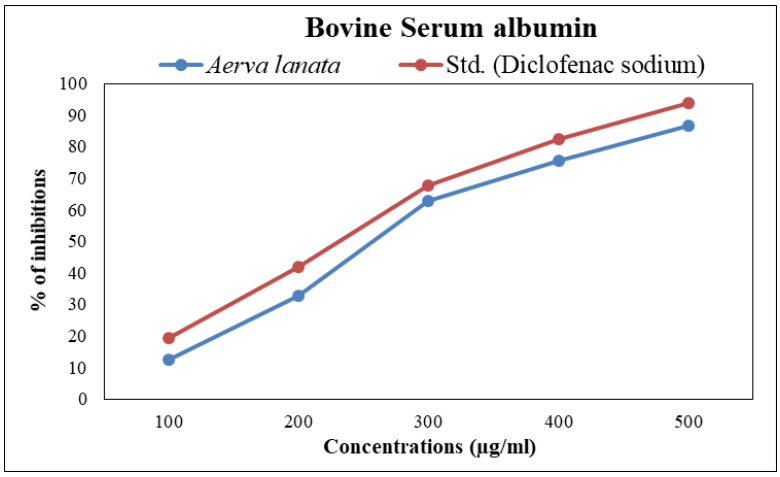


Fig.6: *In vitro* anti-inflammatory activity of *Aerva lanata* leaves (Bovine Serum albumin)

Conclusion

Overall, it can be concluded from the present study that *Aerva lanata* leaves contains rich source of phytochemicals. This study is the first scientific report that provides convincing phytochemicals and anti-inflammatory activity evidence for the relevance of *Aerva lanata* leaves thus providing scientific validity to its traditional consumption by the local populace of south India.

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