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Phytochemical screening, *in vitro* anti-microbial and anti-inflammatory activity of methanolic extract of *aster lanceolatus willd* leaves

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Abstract
The methanolic extract of leaves of *aster lanceolatus* was assessed for its phytochemical screening, in vitro anti-microbial and anti-inflammatory activity. Phytochemical analysis of aster lanceolatus plant extracts revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides, triterpenoids and saponins etc. Since triterpenoids and flavonoids have remarkable anti-inflammatory activity. The methanolic leaf extract of *aster lanceolatus* gave good antimicrobial and anti-inflammatory activity. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation assay, proteinase inhibitory activity, membrane stabilization, heat induced and hypotonicity induced haemolysis at different concentrations. Diclofenac sodium was used as standard drugs. The results obtained in the present study indicate that methanol extracts of aster lanceolatus can be a potential source of anti-inflammatory agents.

Keywords: *Aster lanceolatus,* phytochemical screening, antimicrobial activity, anti-inflammatory activity

1. Introduction

Inflammation is part of the multifaceted biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration [1]. It is the body's attempt to heal itself after an injury; defend itself against foreign invaders, such as viruses and bacteria; and repair damaged tissue. Inflammation is often characterized by redness, swelling, warmth, and sometimes pain and some immobility. There are two types of inflammation: acute and chronic (sometimes called systemic) inflammation. Acute inflammation arises after a cut or scrape in the skin, an infected ingrown nail, a sprained ankle, acute bronchitis, a sore throat, tonsillitis or appendicitis. It is short-term and the effects subside after a few days. Chronic inflammation is long-term and occurs in "wear and tear" conditions, including osteoarthritis, and autoimmune diseases, such as lupus and rheumatoid arthritis, allergies, asthma, inflammatory bowel disease and crohn's disease [2]. Several experimental protocols of inflammation are used for evaluating the potency of drugs. The management of inflammation related diseases is a real issue in the rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal plants [3].

Aster lanceolatus (family: astaracae) is a perennial growing up to two meters tall. The leaves are alternate, long, narrow, widest near the middle, and sometimes irregularly toothed along the edges, ranging up to six inches long. White panicle aster ranges from Maine and south to South Carolina, west Texas and North Dakota. The dried and powdered plant was used as a salve on abrasions. A decoction of the plant was used to dress wounds. Smoke from the crushed blossoms was inhaled in the treatment of nosebleeds. Antibacterial and antifungal properties are reported. A survey of literature indicated no systemic approach has been made to evaluate the anti-inflammatory potential of Aster lanceolatus by invitro method [4, 5]. The present study involves determination of anti-inflammatory activity of albumin denaturation assay, proteinase inhibitory activity, membrane stabilization, heat induced and hypotonicity induced haemolysis.

Materials and methods

Plant material

The leaves of *aster lanceolatus* were collected in fresh condition from our college campus (Pioneer Pharmacy Degree College, Vadodara Gujarat, India). The leaves were dried under shade then ground in to a uniform powder using a blender and stored in polythene bags at room temperature.

Source of microorganisms

The organisms studied, *Escherichia Coli* (MTCC No.40), *Pseudomonas aeroginosa* (MTCC No.424), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Streptococcus mutans* (MTCC No.497), *Bacillus subtilus* (MTCC No.441), *Staphylococcus epidermidis* (MTCC No.9041), *Micrococcus luteus* (MTCC No.106), Saccharomyces cerevisiae (MTCC No.170), *Candida albicans* (MTCC No.183) and *Candida tropicalis* (MTCC No.1000). The organisms were obtained from MTCC Chandigarh and maintain according to specification. Sub culturing was done at the interval of 15 days.

Preparation of extracts

The leaves powder was loaded in to soxhlet extractor and subjected to extraction with methanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator.

Phytochemical analysis [6]
Phytochemical analysis for qualitative detection of alkaloids, glycosides, reducing sugar flavonoids, tannins and saponins was performed by the extracts.

Determination of Antimicrobial Activity
The antimicrobial activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Briefly, Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells were maded in the agar using the stainless steel borer of 8 mm and filled with 100, 200 and 300 µl of plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 72 hours and the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition.

Anti-Inflammatory (Membrane Stability) Activity Assay
HRBC Membrane Stabilization Method [7-9]
The anti-inflammatory activity of various extracts of leaves of *aster lanceolatus* was assessed by in vitro HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with isosaline (0.85 %, Dissolve 8.5 g NaCl in water. Autoclave 15 min at 121°C. Cool to room temperature). To 1mL of HRBC suspension, equal volume of test drug in six different concentrations 100-600 μg/mL, was added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage of haemolysis was calculated then by the formula as given below:

Percent of hemolysis = OD of test/OD of control ×100 .....(1)

The percentage of protection can be hence calculated from the equation as given below:

Percent of protection = 100-OD of test/OD of control ×100 ...(2)

Here "OD of test" is optical density or the test sample's absorbance and "OD of control" is optical density or absorbance of the negative control. Here, the negative control used was Alsever's solution with blood in it and it contained no diclofenac or methanolic extract of the plant material in it. The absorbance of the negative control was found to be 0.225 (Table 3).

Effect on Protein denaturation [10]
Test solution consisting of 1ml of different concentrations of extract / standard drug, Diclofenac sodium of different concentration 100-600 g/ml was mixed with 1ml of egg albumin solution (1mM) and incubated at 27±1 C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 60 °C in a water bath for 10 minutes. After cooling the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate.

The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) X 100/Abs control

Proteinase inhibitory action [10-11]
In brief, the reaction mixture (2 ml) was containing 0.06 mg Proteinase, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample/ standard drug, Diclofenac sodium, of different concentration 100-600 g/ml. The mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X100/ Abs control

Heat induced haemolysis [11, 13]
The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 600 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 60ºC for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 3000 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples.
The percentage inhibition of haemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Hypotonicity-induced haemolysis [12]
Different concentration of extract (100-600µg/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the assay mixtures were incubated at 370c for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection = 100- (OD sample/OD control) x 100

Results and Discussion
Qualitative phytochemical analysis
The present study reveals that plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthraquinones in solvent extracts as shown in Table 1.

Table 1: Phytochemical constituent Present in Extracts

Extract	Alkaloid	Flavanoids	Saponin	Terprnoid	Tannin	Glycoside	Reducing sugar	Volatile oil
Methanolic	+	+	+	+	+	-	-	+

Antimicrobial Activity

The potential sensitivity of the extract was obtained against all the microorganisms tested and the zone of inhibition was recorded. The results obtained were compared against standard antibiotic kanamycin and amphotericin B (and presented below in the tabulation drawn (Table 2).

Table 2: Zone of inhibition of extract and standard

Tested bacteria	Zone of Inhibition ± SD			
	Kanamycin for anti bacterials and Amphotericin B for anti-fungal (30 mcg/ml)	100 mcg/ml	200 mcg/ml	300 mcg/ml
E. coli	25 mm ±0.23	-	12 mm±0.11	14mm±0.85
S. aureus	24 mm±0.11	12mm±0.05	14 mm±0.24	18mm±0.33
P. vulgaris	28 mm±0.16	-	-	15mm±
P. aerouginosa	18 mm±0.32	10mm±0.32	12 mm±0.41	15mm±0.38
B. subtilis	21 mm±0.41	-	-	-
S. epidermidis	22 mm±0.11	-	10 mm±0.72	12 mm±0.56
M. luteus	16 mm±0.56	-	-	10 mm±0.91
S. cerevisiae	20 mm±0.48	-	15 mm±0.31	17 mm±0.25
C. albicans	18 mm±0.52	10mm±0.23	11 mm±0.45	12 mm±0.91
C. tropicalis	21 mm±0.67	13mm±0.87	15 mm ±0.56	17 mm±0.33

(SD: standard deviation n=3)

Membrane stabilization

The HRBC membrane stabilization has been used as a method to study the invitro anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane.

Table 3: Data representing absorbance and % prevention of lysis

Concentration (mcg/ml)	Absorbance	Prevention of lysis (%)
100	0.183	18.66
200	0.175	22.22
300	0.165	26.66
400	0.155	31.11
500	0.144	36.00
600	0.136	39.33
Diclofenac	0.123	45.33
Negative	0.225	00

Inhibition of protein denaturation

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 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 64.70 % was observed at 600 µg/ml. Diclofenac sodium, a standard antiinflammation drug showed the maximum inhibition 61.76 % at the concentration of 100 µg/ml compared with control (Table 4).

Table 4: Effect of extract on protein denaturation

Groups	Concentration (mcg/ml)	Absorbance at 660 nm ± SD	% Inhibition of protein denaturation
Control	-	0.34±0.05	----------
Extract	100	0.24±0.02	29.41
Extract	200	0.22±0.02	34.00
Extract	300	0.19±0.03	44.11
Extract	400	0.16±0.06	52.94
Extract	500	0.13±0.09	61.76
Extract	600	0.12±0.12	64.70
Diclofenac sodium	100	0.13±0.02	61.76

(SD: standard deviation n=3)

Proteinase Inhibitory Action

Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. Extract exhibited significant antiproteinase activity at different concentrations as shown in Table 5. It showed maximum inhibition of 60.60% at 600 µg/ml. Diclofenac sodium showed the maximum inhibition 66.66 % at 100µg/ml.

Table 5: Effect of extract on proteinase inhibitory activity

Groups	Concentration (mcg/ml)	Absorbance at 660 nm ± SD	% Inhibition of protein denaturation
Control	-	0.33±0.16	……..
Extract	100	0.28±0.02	15.15
Extract	200	0.25±0.03	24.24
Extract	300	0.21±0.06	36.36
Extract	400	0.19±0.01	42.42
Extract	500	0.17±0.02	48.48
Extract	600	0.13±0.05	60.60
Diclofenac sodium	100	0.11±0.02	66.66

(SD: standard deviation n=3)

Heat Induced Haemolysis

The extract was effective in inhibiting the heat induced haemolysis at different concentrations. The results showed that extract at concentration 500 and 600µg/ml protect the erythrocyte membrane against lysis induced by heat (Table 6). Diclofenac sodium 100µg/ml offered a protection against damaging effect of heat solution.

Table 6: Effect of extract on heat induced haemolysis of erythrocyte

Groups	Concentration (mcg/ml)	Absorbance at 660 nm ± SD	% Inhibition of protein denaturation
Control	-	0.32±0.05	
Extract	100	0.28±0.03	12.5
Extract	200	0.26±0.01	18.75
Extract	300	0.21±0.08	34.37
Extract	400	0.19±0.05	40.06
Extract	500	0.16±0.04	50
Extract	600	0.12±0.03	62.5
Diclofenac sodium	100	0.10±0.05	68.75

(SD: standard deviation n=3)

Hypotonicity Induced Haemolysis

The results showed that EAME at concentration range of 100-600 µg/ml protect the erythrocyte membrane against lysis induced by hypotonic solution (Table7). Diclofenac sodium (100µg/ml) offered a protection against the damaging effect of hypotonic solution. At the concentration of 600 µg/ml, EAME showed maximum of 64.51% protection, whereas, Diclofenac sodium (100µg/ml) showed 74.19% inhibition of RBC haemolysis when compared with control.

Table 7: Effect of extract on hypotonicity induced haemolysis of erythrocyte

Groups	Concentration (mcg/ml)	Absorbance at 660 nm ± SD	% Inhibition of protein denaturation
Control	-	0.31±0.02	
Extract	100	0.27±0.08	12.90
Extract	200	0.24±0.06	22.58
Extract	300	0.19±0.08	38.70
Extract	400	0.16±0.07	48.38
Extract	500	0.13±0.1	58.06
Extract	600	0.11±0.05	64.51
Diclofenac sodium	100	0.08±0.08	74.19

(SD: standard deviation n=3)

Conclusions

In the present study, results indicate that the methanol extracts of *aster lanceolatus* possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity and stabilized the red blood cells membrane. This study gives on idea that the compound of the plant *aster lanceolatus* can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation.

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