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Evaluation of Anti-Inflammatory Activity on Whole Plant of *Aerva lanata* (L.) by *In-Vitro* Methods

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ABSTRACT

To evaluate the anti-inflammatory events of plant extracts going from *A.lanata* by *In-vitro* strategies**.** The main objectives of the proposed work is to evaluate anti-inflammatory utilization of aqueous (AQEAL) and alcoholic (ALEAL) extracts containing plant of *Aerva lanata* plant in different models of experimental. The Whole plant of *A.lanata* plant powder was made and extracted with 95% alcohol and water (maceration process) to get alcoholic (ALEAL) and aqueous (AQEAL). Preliminary phyto-chemical studies with AQEAL and ALEAL revealed sensational ubiquity of phytoconstitutents of both extracts. Both the extracts of whole plant of *A.lanata* had prevented protein denaturation against heat induced proteolysis.

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INTRODUCTION

Inflammation whatever need the general treatment as chronic time period and the use of medicine can cause numerous unwanted effects, usually with damage of significant organs [1]. But in herbal/traditional medicines, there are several preparations prescribed to treat hyperacidity and arthritics. One of the most vital outgrowth thinking about the general defense reaction opposed to local injury plus diseases, but it frequently develops to painful or pathologically harmful infections involving pharmacologic treatment.

Aerva lanata (L) is a tropical plant which is grown extensively in India, Arabia, Africa, Sri Lanka, Phillipines and Java. It's a perennial weed associated with sensational family Amaranthaceae. *Aerva lanata* may be a medicine that justify remedy a diverse disorder specified helminthic, diabetes, inflammation.

Therein study we have a tendency to aim to research the aqueous extract going from *A. lanata* whole plant for phytochemical composition, membrane stabilization method, protein denaturation method, Heat induced hemolysis [2].

MATERIALS AND METHODS

Diclofenac sodium used to be buying from Sigma-Aldrich Chemical Company USA. Egg albumin, Phosphate buffered pH 6.8, Normal saline was purchased from Himedia Laboratories Private Limited Mumbai, India. Almost all chemical substances used have an analytical grade.

Table 1: Nature and Percentage yield of the extracts

S. No.	Name of the extract	Nature	Color	% yield in g (% w/w)
1.	AQEAL	Sticky	Dark brown	12.5 %
2.	ALEAL	Sticky	Dark green	10%

Plant material

A. lanata used to be self-collected of the natural universal growing in the Seshachalam Forest Area, Chittoor district, Andhra Pradesh, India [3].

Processing of plant

The whole plant of *A. lanata* was collected and washed thoroughly in distilled water and cut into small pieces. The whole plant was shade dried at room temperature. The powder was extracted in distilled water using a Soxhlet apparatus [4]. These extracts were concentrated with a rotary evaporator and dried using lyophilizer.

Phytochemical screening

The powder was screened for the presence of sterols, glycosides, Saponins, Alkaloids, flavonoids, proteins, amino acids, gums and mucilage [5].

Methodology

Preparation of aqueous extract

About 200g of powder of *A.lanata* was taken into a TLC chamber and macerated with 2 liters of distilled water plus 20ml going from ethyl alcohol (preservatives) as 7 days as well as infrequent shaking for 3 times in a day. The marc used to be taken away by way of tracking the extract and the aqueous extract (AQEAL) evaporated at room temperature until the solvent was evaporated [6].

Preparation of alcoholic extract

Almost 100 grams consisting of powder of *A.lanata* was taken into a conical flask and macerated with 400 ml of ethanol for 7 days with occasional shaking for 3 times in a day. The marc was once got rid of by filtering the squeeze its alcohol squeeze (ALEAL) was distilled, alcohol was recollected under distillation, until to obtain a residue of alcohol and that was dried at room temperature [7].

Inhibition of albumin denaturation

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffered pH 6.4 and 2 ml of varying concentrations of extract so that final concentrations. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37^0C$\pm 2^0$C in a BOD incubator for 15mins and then heated at 70^0C for 5mins [8]. Diclofenac Sodium at the final concentration of was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\%\ of\ Inhibition\ = 100\ X\ [V_t/V_c - 1]$$

Where,

V_t = absorbance of test sample,

V_c = absorbance of control.

Membrane stabilization test

Preparation of Red Blood cells (RBCs) suspension

Fresh blood (10 ml) used to be self collected and reassigned to with the heparinzed centrifuged tubes. The tubes have been centrifuged at 3000 rpm for 10 min and have been scrubbed 3 times with equivalent volume of regular saline [9]. The volume of the blood was measured and reconstituted as 10%v/v suspension with normal saline.

Heat induced hemolysis

The reaction mixture (2 ml) consisted of ml of 10% RBCs suspension and 1 ml of varying concentrations of extract and standard drug so that final concentrations. Diclofenac Sodium was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56^oC for 30 min. At the end of the incubation, the tubes were cooled under running tap water [10]. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm.

RESULTS & DISCUSSION

Qualitative Phytochemical analysis

Preliminary phyto-chemical analysis of the AQEAL and ALEAL have revealed the presence of sterols, alkaloids, glycosides, flavanoids, tannins and carbohydrates, saponins, proteins and amino acids in both the extracts [Table 2].

Preparation of alcoholic extract

The AQEAL and ALEAL were examined for his colour and consistency as well as their percent yield used to be calculated with regards to the amount used extraction, stored in air-tight plastic containers in a refrigerator beneath 4^oC [Table 1].

Table 2: Details of Qualitative Phyto-chemical tests

Test	Indication	AQEAL	ALEAL
a)Tests for sterols			
1.Salkowski's test: CHCL3+Conc.H2SO4from sidelong retaining wall the test tube.	Presence of yellow colored ring between junctions of 2 solutions observed which turned into red colored ring after 2 mins	+	+
2.Libermann Burchard's test: CHCL3 + (CH3CO)2O + Conc.H2SO4 from side long retaining wall the test tube.	Presence of violet to blue colored ring between junctions of 2 solutions observed	+	+
b)Tests for Glycosides			
1.Baljet's test : Solution A:1g picric acid in 100ml ethyl alcohol Solution B:10gs NaOH in 100ml distilled water Both solutions combined and 2 to 3 drops of combined solution in 2 to 3 mg of test sample	Formation of orange to deep red color	+	+
2.Keller-killiani test: Extract 50mg was dissolved in 2ml Chloroform. H2SO4 was added to form a layer and the color at interphase recorded	Presence of brown ring at interphase	+	+
3.Borntrager's test: Extract +Dil. H2SO4, boil and filter. filtrate + CHCL3. Separate CHCL3 layer and add dil.NH3	Formation of pink to red color.	+	+
c)Test for Saponins			
Foam test: Extract shaken with water	Presence of foam observed	+	+
d)Test for Carbohydrates			
The extract (50mg) dissolved in 3ml of water and filtered. The filtrate was subjected to the following tests.			
1.Molish's Test: 2ml filterate + α-napthol+1ml H2SO4	Presence of violet ring	+	+
2.Barfoed's test: 1ml filtrate +Barfoed's reagent (heated for 2 mins)	Presence of red precipitate	+	+

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Table 2 continued

Test	Indication	AQEAL	ALEAL
3.Benedict' test: 0.5ml filtrate + Benedict's reagent (heated for 2 mins)	Characteristic colored precipitate	+	+
4.Fehling's test: 0.5ml of extract+0.5ml of both Fehling's A and B solutions heated for 2 mins.	Characteristic colored precipitate	+	+
E)Test For Alkaloids			
The Extract 50mg stirred up along with some milliliter going from HCl and screened, the filtrate used to be well-tired as well tried as well as varied alkaloid testing agent as follows			
1.Mayer's test: Mayer's reagent +Filtrate	Creamy precipitate	+	+
2.Wagner's test: Wagner's reagent + Filtrate	Reddish – Brown precipitate	+	+
3.Hager's test: Hager's reagent + Filtrate	Prominent yellow precipitate	+	+
f)Test for Flavonoids			
1.Ferric chloride test: Extract + FeCl3	Formation of deep green color	+	+
2.Shinoda test : Extract + dil. HCl and Magnesium turning	Presence of red color	+	+
3.Lead acetate test:		+	+
g)Test for Tannins			
1.Ferric chloride test: Extract + FeCl3	Formation of deep green color	+	+
2.Gelatin test		+	+
h)Test for Proteins and amino acids			
1.Millon's test : Filtrate + Millon's reagent	Presence of white precipitate	+	+
2.Ninhydyin test : Filtrate + Ninhydrine solution	Formation of purple color	+	+
i)Tests for Gum, Mucilage			
1.Swelling test : Dry powder + water	Powder swells	+	+
2.Molisch's test: 2 ml filtrate + α-Naphthol + 1ml H2SO4	Presence of Violet ring	+	+

Table 3: *In–vitro* Anti-inflammatory activity of AQEAL & ALEAL by Membrane stabilization method

Test sample	Conc.(μg/ml)	% Protection
Aqueous extract of *Aerva Lanata*	50	23.8
	100	35.6
	200	56.3
	400	78.6
	800	104.4
Alcoholic extract of *Aerva Lanata*	50	27.07
	100	42.9
	200	71.3
	400	94.9
	800	119.4
Effect of Diclofenac sodium (std .drug)	50	22.9
	100	31.2
	200	96.4
	400	119.7
	800	132.8

Table 4: AQEAL & ALEAL by Protein denaturation method

Sample	Conc.(μg/ml)	% Protection
Aqueous extract of *Aerva Lanata*	50	15.8
	100	31.08
	200	75.3
	400	82.9
	800	129.3
Alcoholic extract of *Aerva Lanata*	50	17.5
	100	45.1
	200	76.5
	400	104.9
	800	130.2
Effect of Diclofenac sodium (std .drug)	50	5.2
	100	13.1
	200	31.6
	400	63.9
	800	128.1

Anti-inflammatory effect of AQEAL and ALEAL have been planned considerably by testing varied *in-vitro* parameters. The consequence of AQEAL on membrane stabilization at abundant dose ranges 50, 100, 200, 400 and 800μg/ml showed significant protective cover against RBC membrane damage found to be 23.8%, 35.6%, 56.3%, 78.6% and 104.4 % respectively.

The effect of ALEAL on membrane stabilization at dose intervals 50, 100, 200, 400 and 800 μg/ml had shown vital protective covering against RBC membrane damage found to be 27.07%, 42.9%, 71.3%, 94.9%, and 119.4% respectively.

The effect of Diclofenac sodium on membrane stabilization at abundant recall dose levels 50, 100, 200, 400 and 800μg/ml had shown vital protection against RBC membrane damage found to be 22.9%, 31.2%, 96.4%, 119.7% and 132.8% respectively [Table 3].

The effect going from AQEAL on hold up of protein denaturation at poles apart dose levels 50, 100, 200, 400 and 800 μg/ml had shown significant protection against denaturation of proteins found to be 15.8%, 31.08%, 75.3%, 82.9% and 129.3% respectively.

The effect going from ALEAL on hold up of protein denaturation abundant dose intervals 50, 100, 200, 400 and 800 μg/ml had shown significant protective covering opposed to denaturing of proteins found to be 17.5%, 45.1%, 76.5%, 104.9% and 130.2%respectively [Table 4].

The results of Diclofenac Sodium on delay of proein denaturing at abundant dose levels 50, 100, 200, 400 and 800 μg/ml had shown vital protective cover opposed to denaturing going from protein found to be 2.2%, 13.1%, 31.6%, 63.9% and 128.1% respectively.

CONCLUSION

Therefore, it can be concluded that AQEAL & ALEAL of whole plant possessed with anti-inflammatory activity. Preliminary phytochemical analysis the AQEAL plus ALEAL submit to unconcealed sensational ubiquity of sterols, alkaloids, glycosides, flavanoids, tannins and carbohydrates, saponins, proteins and amino acids in both the extracts. Both the extracts of whole plant of *A.lanata* had prevented protein denaturation against heat induced proteolysis. Both the extracts of whole plant of *A.lanata* had prevented membrane stabilization against heat induced hemolysis. Above two inferences strongly suggested that Anti-inflammatory activity of both the extracts exhibited by *In-vitro* methods. However, AQEAL of whole plant has produced slightly better anti-inflammatory activity than the ALEAL of whole plant.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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