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Invitro-Anti Inflammatory and Anti Oxidant Activity of Euphorbia Nivulia

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ABSTRACT

The drug discovery is very important Process. At the time of invention of new molecule of there is necessity of related fields like medicine, bio technology, chemistry and pharmacology. Plant remains the most common source of for a drug. In recent Years, the Production of natural products are increased in pharmaceutical companies due to minimal side effects. Herbal medicines are the oldest remedies known to mankind. India has one of the well known histories for the use of medicinal herbs[1]. The present study was to evaluate the In-Vitro Anti Inflammatory and Anti Oxidant activity of *Euphorbia Nivulia* , (family: *Euphorbiaceae*)in various exracts. Anti inflammatory activity was studied using albumin denaturation assay , membrane stabilization assay at different concentrations. Aspirin was used as standard drug & anti oxidant activity was studied by DPPH radical scavenging activity by photometric assay using Rutin as standard. Results showed that the present study indicates that *phytochemicals* present in *Euphorbia nivulia* in various extracts may be responsible for Anti inflammatory and Anti oxidant activity.

Keywords: *Euphorbia Nivulia*,Anti inflammatory, Anti oxidant.

I. INTRODUCTION

Inflammation is consider as a primary physiologic defence mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens, or other noxious stimuli.

An uncontrolled and persistent inflammation may act as an aetiology factor for many of these chronic illness. Although it is a defence mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases. Currently used synthetic anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs are associated with fewer side effects is necessary from medicinal plants origin[2].

Free radicals which have one or more unpaired electrons (superoxide, hydroxyl, peroxyl) are produced in normal or pathological cell metabolism and the compounds that can scavenge free radicals have great potential in amelio-rating the diseases and pathological cells.

Anti oxidants play on important role to protect the human body against damage by reactive oxygen species. Free radicals or reactive oxygen from the respiratory chain as a result occasional challenges. These free radicals are the main culprits in lipid Peroxidation. Plants congaing bioactive compounds have been reported to possess strong antioxidant properties. In many inflammatory disorders there is excessive activation of phagocytes, production of O^{2-} ,OH radicals as well as non free radicals

species(H_2O_2)(Gilham et al.,1997), which can harm severely tissues either by powerful direct oxidizing action or indirect with hydrogen peroxide and –OH radical formed from O^{2-} which initiates lipid Peroxidation resulting in membrane destruction.Tissue damage than provokes inflammatory response by production of mediators and chemotactic factors (Lewis,1989). The reactive Oxygen species are also known to activate matrix metallo proteinase damage seen in various arthritic tissues (Cotran et al.,1994)[2].

II. METHODS AND MATERIAL

The plant material was collected from young matured plant from the western hills village belt around Srivilliputtur and authenticated by the botanist. The whole plant was collected in bulk , washed to remove adhering dust, dried under shade and pulverised in a mechanical grinder. The powder was passed through sieve number No: 40 and used for further studies.

Preparation of the Extract:

The freshly collected whole plant were chopped into pieces and shade under dried at room temperature (32- 35°C) to constant weight for 5 days. The plant material was extracted with Methanol by hot and cold maceration method. The extract was concentrated and dried under reduced pressure.

Chemicals and reagents:

1% aqueous solution of Bovine serum, Methanol, 1N Hcl, Normal saline, Aspirin, and Human blood. Reagents:0.4mM DPPH.

Phytochemical Evaluation[3]

Methanolic extract of Euphorbia Nivulia was studied for its phytoconstitutents such as alkaloids, flavonoids, glycosides, tannins, steroids, carbohydrates and other constituents using phytochemical tests.

Assesment of In-vitro anti inflammatory activity

Inhibition of albumin denaturation[4]

Method of *mizushima* et al was followed the reaction mixture was consisting of test extract at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted by using small amount of 1N Hcl. The sample were incubated at 37°C for 20 min and then heated at 57°C for 20 min. After cooling the samples , the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percentage inhibition of protein denaturation was calculated as follows.

Percentage inhibition =

$\{Abs_{control} - Abs_{sample}\} \times 100/Abs_{control}$

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

Fresh human whole blood (10ml) was collected and transferred to the heparinised centrifuged tubes. The tubes were centrifuged tubes. The tubes were centrifuged at 3000rpm for 10 min and were washed three times with equal volume of nasal saline. The volume of the blood was measured and reconstituted as 10 % v/v suspension with normal saline.

Heat induced hemolysis

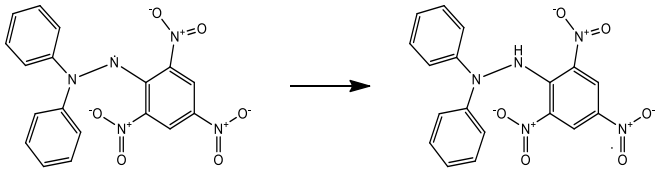
The reaction mixture (2ml) consisted of 1 ml test drug solution and 1 ml of 10 % RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the end of the incubation, the tubes were cooled under running tape water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates. Percent membrane stabilization activity was calculated by the formula mentioned above.

In-vitro antioxidant activity[5]

DPPH photometric assay

Principle

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-Diphenyl -2- picryl hydrazine. A coloured complex is formed which can be measured colorimetrically at 518 nm.



Procedure

The effect of extract on DPPH radical was assayed using the method of Mensor *et al*., (2001). A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging activity(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical + methanol;

A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

III. RESULTS AND DISCUSSION

The yield of the methanolic extract of *Euphorbia Nivulia* showed the phytochemical studies indicates the presence of alkaloids , flavonoids, saponins, glycosides and tannins.

Effect of methanolic extract of *Euphorbia Nivulia* on albumin denaturation, membrane stabilization activity. Table- 01

S.No	Conc (µg/ml)	Albumin Denaturation	Membrane Stabilization
01.	50	23.72±1.48	19.84±0.12
02.	100	33.82±1.14	22.14±0.65
03.	200	48.87±1.12	29.44±0.32
04.	400	65.43±1.11	32.14±.015
05.	600	75.54±1.14	45.32±1.22
06.	Aspirin	80.21±0.12	72.56±0.52

Values are represent in the results are mean ± SD of the three replicates

Effect of aqueous extract of *Euphorbia Nivulia* on albumin denaturation, membrane stabilization activity .Table- 02

S.No	Conc (µg/ml)	Albumin Denaturation	Membrane Stabilization
01.	50	13.70±1.38	17.24±0.12
02.	100	23.92±1.16	20.14±0.72
03.	200	38.87±1.02	29.44±0.32
04.	400	45.40±1.11	32.14±.010
05.	600	55.50±1.14	42.12±1.20
06.	Aspirin	60.21±0.17	52.06±0.22

Values are represent in the results are mean ± SD of the three replicates.

In-Vitro Anti Oxidants Activity

DPPH Photometric Assay

The percentage of DPPH radical scavenging activity of various extracts of plant 1 *a*re presented in Table. The ethanolic extract of *Euphorbia nivulia* was found to be more effective than ethyl acetate extract. The DPPH radical scavenging activity of the extract increases with increasing concentration. The IC_{50} of

the ethanolic extract of *Euphorbia nivulia* and Rutin were found to be 280µg/ml and 480µg/ml respectively

Table Effect of various extracts of *Euphorbia nivulia* on DPPH assay. Table- 03

Concentration (µg/ml)	% of Activity (±SEM)*		
	Ethyl acetate Extract	Ethanol Extract	Standard (Rutin)
100	17.45 ± 0.16	38.50 ± 0.45	18.28 ± 0.15
200	29.40 ± 0.18	48.65 ± 0.15	22.28 ± 0.47
400	38.42 ± 0.11	65.68 ± 0.21	52.14 ± 0.32
800	41.23 ± 0.10	71.18 ± 0.26	71.40 ± 0.40
	IC_{50}=1220 µg/ml	IC_{50}=280 µg/ml	IC_{50}=480 µg/ml

*All values are expressed as mean ± SEM for three determinations

IV. CONCLUSION

The present study revealed the the methanolic extract of *Euphorbia Nivulia* shows In- vitro anti inflammatory and anti oxidant activity. *Euphorbia nivulia* may be consider as valuable plant in both ayurvedic and modern drug development areas of its versatile medicinal uses. The presence of alkaloids, flavonoids and saponins may responsible for the activity. Further investigations are required to find active component of the extract and to confirm the mechanism of action. Hence it can be taken for the further research and the phytoconsituents if isolated will provide a useful drug to human community

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