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EVALUATION OF *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *Alstonia Scholaris* FLOWERS EXTRACT

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ABSTRACT

The aim of the present study was to evaluate the invitro antioxidant activity of *Alstonia Scholaris* flowers extract by different methods like DPPH radical scavenging, assay, Nitric oxide radical assay and phosphomolybdnum assay method. Invitro anti-inflammatory activity of *Alstonia Scholaris* flowers extract was also evaluated by Protein Denaturation and HRBC method. *Alstonia Scholaris* flowers extract has exhibited significant antioxidant and anti inflammatory activities.

Key words: *Alstonia Scholaris*, Antioxidant, Anti inflammatory.

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INTRODUCTION

The species *Alstonia Scholaris* (L) R.Br was originally named *Echites Scholaris* by Linnaeus in 1767. *Alstonia Scholaris* (Apocynaceae) commonly called black board tree, Ditabark an ever green tropical tree native to Indian subcontiennt. Traditional ueses of *Alstonia*[1-3] as stimulant, carminative, stomachic, bitter tonic, astringent, febrifuge and Ethanolic extract of bark was traditionally used as Anti leishmanial activity. Local natives of East Godavari District habitants were using the decoction of *Alstonia Scholaris* as Anti rheumatic and also as pain killer. The present study is to investigate and explore the possible invitro antioxidant and invitro anti inflammatory activity of ethanolic extract of *Alstonia Scholaris* flowers.

MATERIALS & METHODS

Plant Collection:

Alstonia Scholaris flowers were collected from Aditya Gardens situated in Surampalem, East Godavari District, A.P. The plant was authenticated by Dr. T. Raghuram, Taxonomist, SRVBSJB Maharani College, Peddapuram.

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Preparation of Extract

The flowers of *Alstonia Scholaris* were dried under shade for 1 week and then powdered coarsely and macerated with ethanol for 3 days. The filtrate is concentrated with distillation process, after completion of the process the concentrate crude extract is kept in china dish and stored in vacuum desiccator.

Assessment of Antioxidant Activity

Antioxidant activity of *Alstonia Scholaris* flowers extract was determined by DPPH, Nitric Oxide and phosphomolybdenum assay.

DPPH Method

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol (10). This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The Percentage inhibition of

Alstonia Scholaris flowers extract was found to be 62.9, 70.1, 80 and 83.90 percentage at 50, 100,300 and 500 µg / ml. The corresponding IC_{50} values was displayed in Table 1 and the values were compared with standard Ascorbic acid. To ensure the capacity of test extract in DPPH scavenging property, the following was the equation

$$\text{DPPH Scavenged (\%)} = \frac{(A0-A1)}{A0} \times 100$$

Where A0 = absorbance of blank solution,
A1 = test extract absorbance

Nitric Oxide method

Nitric oxide method can be determined by Gresis reaction[4 - 5] Nitropruside releases Nitric Oxide in aqueous solution at pH 7.2. Nitric Oxide reacts with oxygen and produces Nitrate and Nitrite. Scavenger of Nitric Oxide compete with oxygen which ultimately decreases Nitric Oxide production. 5mM of sodium Nitropruside was mixed with various concentrations of test extracts 50, 100, 300 & 500 µg / ml added to methanol and then incubated for 120 minutes at 30^0C. The above samples were reaction with 0.5ml of Gresis reagent (1% Sulphanalamide, 2% H_3PO_4 and 0.1% Napthyl ethylene diamine, dihydrochloride and the absorbance was measured 546nm. The percentage of inhibition was calculated using the formula.

$$\text{Nitric Oxide Scavenged (\%)} = \frac{1(A0-A1)}{A01} \times 100$$

Where A0 = absorbance of blank solution,
A1 = test extract absorbance

Phosphomolybdnum Method

This method completely depends upon the reduction of MO (V1) to MO (V) through the extract and then the green phosphate / MO (V) is formed at pH < 7. To 0.3 ml of *Alstonia Scholaris* extract (50, 100, 300 and 500 µg / ml) added 3ml of reagent (0.6 M of H_2SO_4 and 2g mM of Na_3PO_4 and 4mM of Ammonium molybdate was added[6]. The test tubes were incubated for 90 minutes at 95^0C and the absorbance was measured at 695nm by Spectrophotometer.

Assessment of Invitro Anti-inflammatory Activity
Inhibition of Protein Denaturation method

Denaturation of proteins is a well-documented cause of inflammation. Diclofenac sodium, phenylbutazone and salicylic acid, etc., have shown dose dependent ability to the induced protein denaturation.

Procedure

Test solution (0.5ml) consist of 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml of test sample of different concentrations (50µg / ml, 100 µg / ml, 300 µg / ml, and 500 µg / ml). Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml of distilled water.

Product control solution (0.5ml) consist of 0.45ml of distilled water and 0.5ml of test samples of different concentrations (50µg / ml, 100 µg / ml, 300 µg / ml, and 500 µg / ml). Standard solution (0.5ml) consist of 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml of different concentrations (100 and 200µg / ml of Diclofenac sodium).

All the above solutions were adjusted to pH 6.3 using 1 N Hcl. The samples were incubated at 37^0Cfor 20 minutes and the temperature was increased to keep the sample at 50^0C for 3 minutes. After cooling 2.5ml of phosphate buffer was added to the above solutions, the absorbance was measured using UV Spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated as

$$\text{\% inhibition of protein denaturation} = 100 - \frac{[\{OD\ of\ test\ solution - OD\ of\ product\ solution\}]}{OD\ of\ test\ control} \times 10$$

The control represents 100% protein denaturation and the results were compared with standard Diclofenac sodium

HRBC Membrane stabilization Method

The erythrocyte membrane resembles to lyosomal membrane and as such the effect of drug on the stabilization of the erythrocyte could be extrapolated to the stabilization of lyosomal membrane. Therefore as the membrane stabilizers it interferes with the release and / or action of mediators like histamine, serotonin, prostaglandins and leukotrienes which are responsible for inflammation. The prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of antiinflammatory activity.

Preparation of HRBC suspension

Fresh whole blood was collected and mixed with equal volume of sterilized Alsever solution (2% Dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 RPM for 10 minutes and packed cells were washed 3 times with isosaline (0.85% at pH 7.2) the volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.

Procedure (Hypotonic Solution – Induced Haemolysis)

The reaction mixture (4.5ml) consists of 2 ml of hyposaline (0.25% w/v NaCl), 1ml of 0.15M phosphate buffer (pH 7.4) and 1ml of test solution (50µg / ml, 100

µg / ml, 300 µg / ml, and 500 µg / ml) in isosaline 0.5ml of 10% HRBC in insulin was added. For test control, 1ml of distilled water used instead of hyposaline (to produce 100% haemolysis) whole product control lacked red blood cells. The mixtures were incubated at 37^0C for 30 minutes and centrifuged at 3000 RPM for 20 minutes, Diclofenac Sodium was used as a reference standard drug. The haemoglobin content in the suspension was estimated using Spectrophotometer at 560nm. Percentage membrane stabilizing activity was calculated

% Membrane stabilization = 100 – [(OD of test sample / OD of control) x 100]

RESULTS

The results of antioxidant values expressed as IC_{50} against various free radicals are shown in Tables I, II and III and Figures 1, 2 and 3 respectively. The calculated IC_{50} values for test extract and reference standard Ascorbic acid were determined.

Antioxidant Activity: DPPH Method

The percentage inhibition of *Alstonia Scholaris* flowers extract was found to be 62.9, 70.1, 80 and 83.90 percentages at 50, 100, 300 & 500 µg/ml. The corresponding IC_{50} values are expressed in Table I and Fig 1.

Nitric oxide method

The percentage inhibition using nitric oxide scavenging method ranged from 67.8 to 89.5 % and the values are compared with standard Ascorbic acid. The results are displayed in Table II and Figure 2.

Phosphomolybdenum Method

The antioxidant capability of *Alstonia Scholaris* flower extracts are shown in Table III and Figure 3. The absorbance is increased with an increase in the concentration at 695nm.

Anti inflammatory Activity

The invitro antiinflammatory activity was performed by using inhibition of protein denaturation and HRBC membrane stabilization methods. The invitro antiinflammatory activity by protein denaturation method of *Alstonia Scholaris* flower extract at 500 µg / ml and the percentage inhibition was 80.4 and the result was compared standard Diclofenac Sodium. Similarly by adopting HRBC method the *Alstonia Scholaris* flower extracts at 500µg / ml concentration produced 81.5% inhibition and the results was compared with standard Diclofenac sodium which produced percentage membrane stabilization at 200µg / ml was 93.80%.

Table 1. Antioxidant activity of *Alstonia Scholaris* flowers extract by DPPH method

			Ascorbic Acid		
Concentration µg / ml	**Flower Extract % of inhibition**	**IC_{50} µg / ml**	**Concentration µg / ml**	**% of inhibition**	**IC_{50} µg / ml**
50	62.9 ± 0.42	1.53	50	63.80 ± 0.23	1.95
100	70.1 ± 0.33		100	71.50 ± 0.31	
300	80.0 ± 0.20		300	82.30 ± 0.15	
500	83.9 ± 0.45		500	89.50 ± 0.18	

Values are expressed as Mean ± std, n = 3

Table 2. Antioxidant activity of *Alstonia Scholaris* flowers extract by Nitric Oxide Scavenging Method

			Nitric Oxide		
Concentration µg / ml	**Flower Extract % of inhibition**	**IC_{50} µg / ml**	**Concentration µg / ml**	**% of inhibition**	**IC_{50} µg / ml**
50	67.8 ± 0.45	1.86	50	71.60 ± 0.45	2.45
100	73.5 ± 0.22		100	79.80 ± 0.60	
300	84.6 ± 0.90		300	90.20 ± 0.80	
500	89.5 ± 0.66		500	94.60 ± 0.70	

Values are expressed as Mean ± std, n = 3

Table 3. Antioxidant activity of *Alstonia Scholaris* flowers extract by Phosphomolybdenum Method

Concentration µg / ml	Flower Extract % of inhibition	Concentration µg / ml	Absorbance of Ascorbic Acid
50	0.08 ± 0.005	50	0.24 ± 0.006
100	0.127 ± 0.008	100	0.37 ± 0.004
300	0.15 ± 0.003	300	0.42 ± 0.007
500	0.19 ± 0.002	500	0.48 ± 0.005

Values are expressed as Mean ± std, n = 3

Table 4. Antiinflammatory activity of *Alstonia Scholaris* flowers extract by Inhibition of Protein Denaturation Method

Concentration µg / ml	Percentage Inhibition of protein denaturation ± Sem		
		Diclofenac Sodium	
	Test Extract	Concentration µg / ml	
50	35.0 ± 0.38	-	-
100	53.6 ± 0.25	-	-
300	74.2 ± 0.70	100	85.6 ± 0.04
500	80.4 ± 0.80	200	94.3 ± 0.08

Values are expressed as Mean ± std, n = 3

Table 5. Antiinflammatory activity of *Alstonia Scholaris* flowers extract by HRBC Membrane Stabilization Method

		Diclofenac Sodium	
Concentration µg / ml	Test Extract concentration µg / ml	Concentration µg / ml	
50	54.2 ± 0.15	-	-
100	61.7 ± 0.05	-	-
300	70.8 ± 0.07	100	87.50 ± 0.06
500	81.8 ± 0.18	200	93.80 ± 0.04

Values are expressed as Mean ± std, n = 3

Figure -1

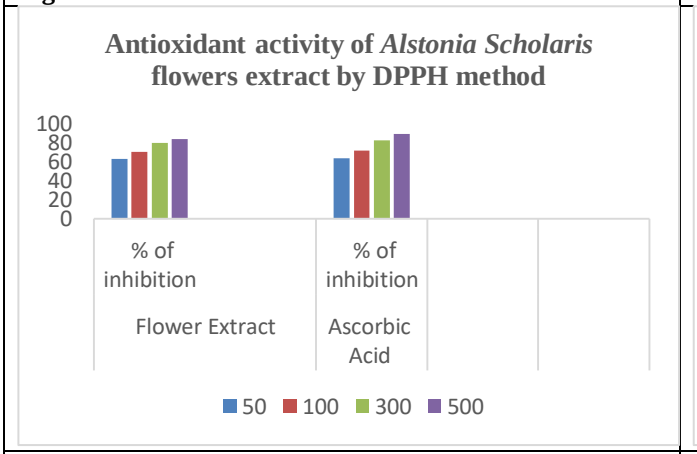


Figure -2

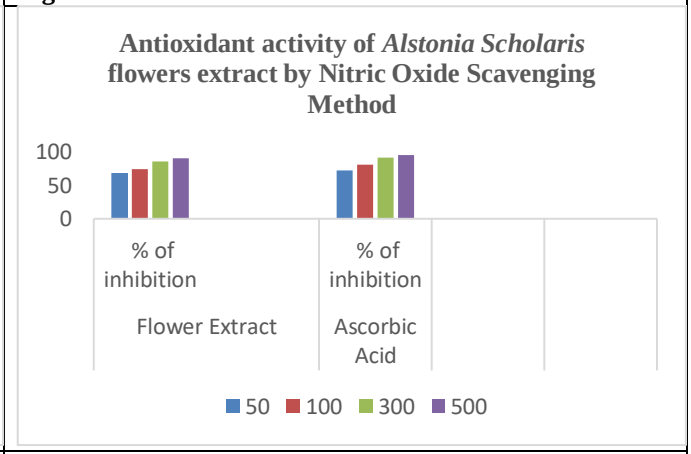


Figure -3

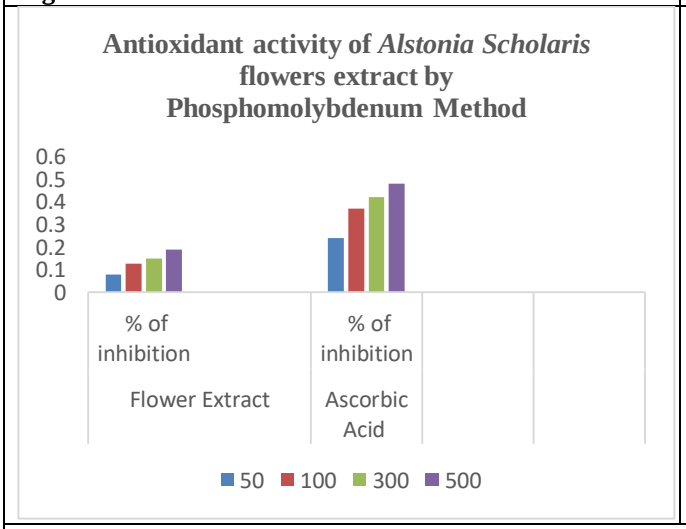


Figure -4

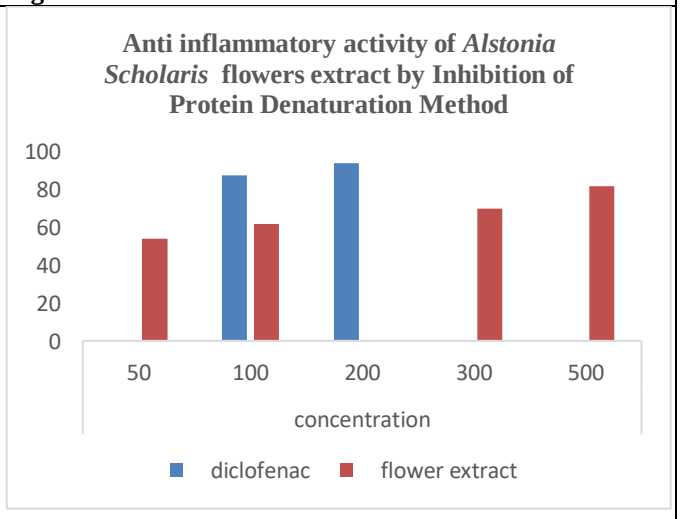
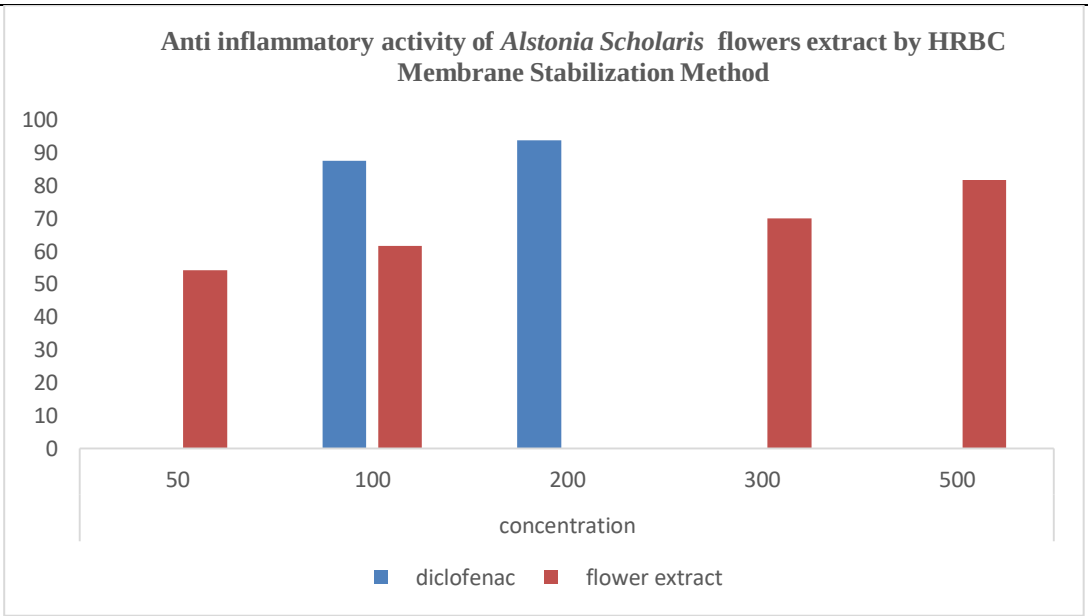


Figure -5



DISCUSSION

In the present study, the antioxidant activity was carried out by DPPH, Nitric oxide and phosphomolybdenum methods. The DPPH method is most versatile method for testing scavenging activity of *Alstonia Scholaris* flowers extract. The scavenging ability of DPPH depends upon inhibition of lipid peroxidation. The entire test is based on DPPH compound[7], the compound consists of odd electrons which are responsible for decolonization of antioxidants which can be measured in the absorbances. The mechanism of nitric oxide method is the formation of nitric oxide through nitroprusside which reacts with oxygen to form nitrite and nitrate[8]. This formation can be incubated by antioxidants by competing with oxygen. From the obtained results the flower extract of *Alstonia Scholaris* showed greater percentage of inhibition. The Phoshomolybdenum assay is associated with the mechanism in reduction of MO (V1) to green phosphate MO (V)[9]. The antiinflammatory activity was performed by protein denaturation and HRBC membrane stabilization methods. From the results obtained it was proved that the *Alstonia scholaris* flower extract exhibited significant antiinflammatory activity and the results were compared with standard reference drug Diclofenac sodium.

CONCLUSION

The results of the present study demonstrate that the *Alstonia Scholaris* flower extract exhibited good invitro antioxidant and antiinflammatory activities. These significant activities suggest valuable indications about further studies to be taken up to evaluate the biological activities.

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