ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

In vitro analysis of antidiabetic and anti-inflammatory activities of selected medicinal plants

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Abstract

The present study was designed to investigate the inhibitory effect on α-amylase of the aqueous bark and leaf extracts of three medicinal plants viz., *Polyalthia longifolia* Benth. and Hook., *Solanum torvum* Swartz. and *Thespesia populnea* (L.) Soland. ex Correa. Fresh leaves and barks of all the three plants were collected, shade dried and aqueous extracts were prepared. For assessing antidiabetic properties, inhibitory effect on α-amylase activity was carried out using DNSA method. For anti-inflammatory studies, HRBC membrane stabilization activity was done. Results in our study revealed that leaf and bark extracts of all the plants showed antidiabetic and anti-inflammatory activities. These extracts caused a significant decrease in releasing maltose during the experiment. *Solanum torvum* leaves showed 53.58% of inhibition over control and the value for *Thespesia populnea* were found to be 46.60% at the concentration of 200 µg. Comparatively *Polyalthia longifolia* showed more inhibitory activity among the three plants investigated as it was evident from the IC_{50} value (105 µg). These extracts caused a significant decrease in the lysis of RBC during the experiment. *Thespesia populnea* showed highest activity (73.02%) among the three plants studied. The IC_{50} values suggest that the leaf extract of *Thespesia populnea* was more effective in inhibiting HRBC hemolysis (IC_{50} was 137.5 µg) than *Solanum torvum* and *Polyalthia longifolia*. The present findings suggest that, the aqueous extracts showed a significant inhibitory effect on α-amylase and heat induced hemolysis of RBC. Further research could be focused in future to identify the phytochemical constituents responsible for such activities and their exploration in the pharmaceutical industries.

Keywords: Anti-inflammatory, α-amylase, Anti diabetic, *In vitro*, Hemolysis.

1. INTRODUCTION

Diabetes mellitus is an unbearable and habitually life threatening disorder with increasing incidence throughout the world. There is a firm rise in the rate of occurrence of Diabetes [1]. Insulin is the main regulator of sugar in the bloodstream. In the case of diabetes, the human body is either unable to produce enough insulin or unable to use its own insulin as well as it should. This causes sugar to build up in blood. Diabetes can cause serious health complications including heart disease, blindness, kidney failure, and lower-extremity amputations. It is the seventh leading cause of death in the United States. In 2014 the global prevalence of diabetes was estimated to be 9% among adults aged above 18 years. More than 80% of diabetes deaths occur in low- and middle-income countries [2]. WHO projects that diabetes will be the 7th leading cause of death in 2030 [3]. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India [4]. It is predicted that by 2030, diabetes mellitus may afflict up to 79.4 million individuals in India.

There is an increase demand in industry to use substitute approaches to treat diabetes, such as plant-based medicines, is also due to the side effects related with the

use of insulin and oral hypoglycaemic agents [5]. It is estimated that more than 200 species of plants exhibit anti-diabetic properties [6]. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the globe [7]. More than 400 herbal plants with glucose-lowering likely are known [8]. Herbal plants have been used for several years by different cultures in the region of the world for the cure of diabetes [9].

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, irritants, injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions [10]. It is triggered by the release of chemical mediators from injured tissue and migrating cells. The commonly used drugs for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs). These drugs block COX-1 and COX-2 enzyme activity preventing the prostaglandin production.

NSAIDs have several adverse effects especially gastric irritation leading to formation of gastric ulcers. Long term uses of these drugs cause adverse side effects and damage human biological system such as liver, gastrointestinal tract, etc. So there is a need for the new safe, potent, non toxic or less toxic anti-inflammatory drug. Plant medicines are of great importance in the primary healthcare in many developing countries. The anti inflammatory drugs act as the lysosomal membrane stabilizing agents.

In the present investigation, the antidiabetic and anti inflammatory properties of three plants viz., *Polyalthia longifolia* Benth. and Hook., *Solanum torvum* Swartz. and *Thespesia populnea*. (L.) *Soland. ex Correa* were evaluated. The aqueous extracts of leaf and bark were tested for α-amylase inhibition activity and erythrocytes membrane stabilization ability.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant materials

The fresh leaves and stem barks of three medicinal plants such as *Polyalthia longifolia* Benth. and Hook., *Solanum torvum* Swartz. and *Thespesia populnea* (L.) Soland. ex Correa. were collected locally and authenticated by the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

2.2. Preparation of plant extracts

The shade dried leaves and stem barks were powdered mechanically and stored in an air tight container. The aqueous extracts were prepared by successive maceration of the powder (10.0g) at room temperature with distilled water (100mL) in mechanical shaker for two days. The final extracts obtained were filtered and the filtrate was concentrated to dryness under controlled temperature 40-50°C. The extracts were preserved in refrigerator till further use [11].

2.3. Antidiabetic activity

2.3.1. Inhibition assay for α-amylase activity (DNSA method)

Inhibitory effect on α-amylase activity was studied using DNSA method [12]. 10 mg of aqueous extract was taken and dissolved in 100 ml of ddH_2O which is used as stock solution with the concentration of 100 µg/ml. Different aliquots of extracts (0.5 mL, 1.0 mL, 1.5 mL and 2.0 mL (containing 50µg, 100µg, 150µg and 200µg respectively) were taken in separate tubes. All the tubes were added with 1.0mL of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution (unit/ml) and made up to 5.0 mL with buffer and were incubated for 10 minutes at 25°C. After pre-incubation1.0 mL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added to each tube. This reaction mixture was then incubated for 10 minutes at 25°C. Then 1.0 mL of DNSA colour reagent was added to stop the reaction.

These test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally this reaction mixture was made up to 10 ml using distilled water following which absorbance was measured at 540nm [13].

% inhibition = (A_{540} control – A_{540} Extract)/A_{540} control × 100

Where A_{540} control is the absorbance without sample, A_{540} extract is the absorbance of sample extract/standard.

2.4. Anti-inflammatory activity

2.4.1. Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10mL) was collected and transferred to the heparinized centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline [14, 15].

2.4.2. Membrane stabilization test - Heat induced hemolysis

The reaction mixture consisted of different aliquots of plant extracts (containing 50µg, 100µg, 150µg, 200µg and 250 µg) and 1.0 mL of 10% RBCs suspension was made. Instead of test sample, only saline was added to the control test tube. All the tubes were made up to 5.0mL with isosaline and incubated in water bath at 56ºC for 30min except the blank. Individual blanks were prepared to nullify the impact of absorbance by plant extracts. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 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. Percent membrane stabilization activity was calculated by the formula mentioned below [16].

% inhibition = (A_{560} control – A_{560} Extract)/ A_{560} control× 100

Where A_{560} control is the absorbance without sample, A_{560} extract is the absorbance of sample extract/standard.

3. RESULTS AND DISCUSSION

3.1. Antidiabetic activity

Four concentrations of leaf and bark extracts of three medicinal plants viz., *Polyalthia longifolia* Benth. and Hook., *Solanum torvum* Swartz. and *Thespesia populnea* (L.) Soland. ex Correa. were used to study whether the impact on α-amylase was dose dependent or not. (Plate – 1)

Results in our study revealed that leaf and bark extracts of all the plants showed inhibitory effect on α-amylase activity. These extracts caused a significant decrease in releasing maltose during the experiment (Tables 1, 2 & 3). It was evident from the table that the aqueous extracts were found to be potent inhibitors of α-amylase compared to control. The bark extract was found to be more potent than the leaf extract in the case of *Polyalthia longifolia* which was showing the higher percentage of inhibition for bark (79.40%) at the concentration of 200 µg than the leaf extract (75.28%) at the same concentration.

In contrast to *Polyalthia longifolia*, *Solanum torvum* and *Thespesia populnea* plants exhibited more inhibitory activity with the leaf extract when compared to the bark. Both the plants showed almost similar activity of α-amylase inhibition (Table 2 & 3). *Solanum torvum* leaves showed 53.58% of inhibition over control and the value for *Thespesia populnea* were found to be 46.60% at the concentration of 200 µg. (Figures 1, 2 &3). Comparatively *Polyalthia longifolia* showed more inhibitory activity among the three plants investigated as it was evident from the IC_{50} value (Plate – 2).

The IC_{50} values suggest that the bark extracts of *Polyalthia longifolia* was more effective in inhibiting α-amylase (IC_{50} was 105 µg). *Thespesia populnea* bark showed the minimum activity with an IC_{50} value of 222.5 µg.

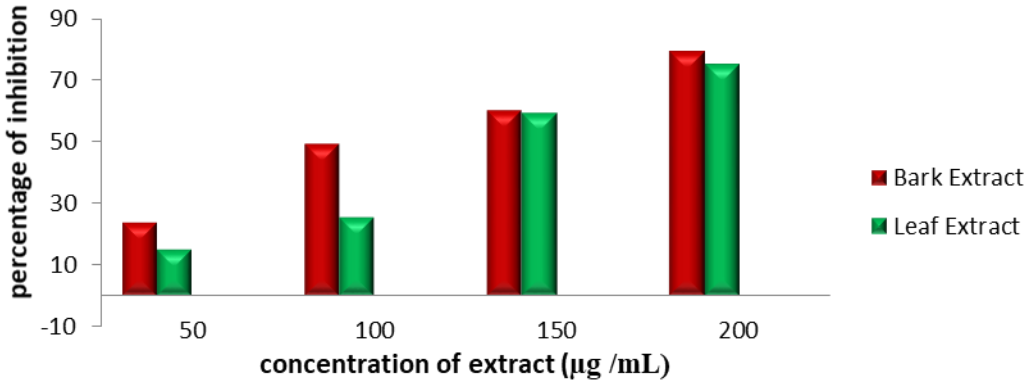


Fig. 1. α-amylase inhibition activity of bark and leaf extracts of *Polyalthia longifolia*

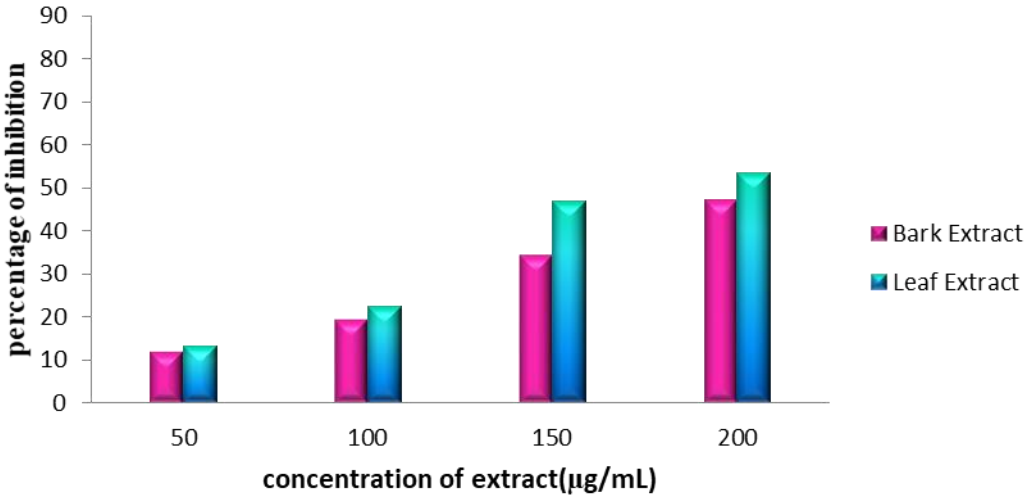


Fig. 2. α-amylase inhibition activity of bark and leaf extracts of *Solanum torvum*

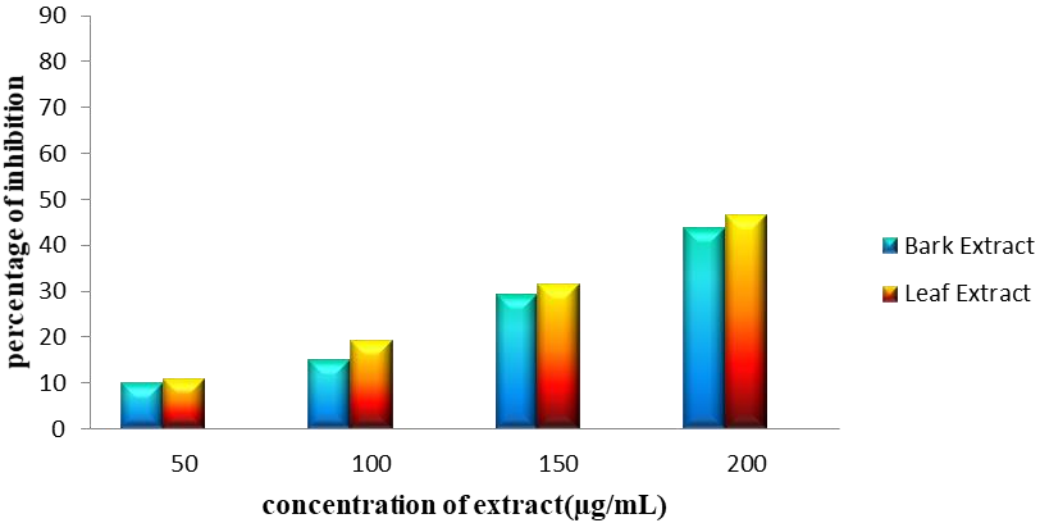


Fig. 3. α-amylase inhibition activity of bark and leaf extracts of *Thespesia populnea*

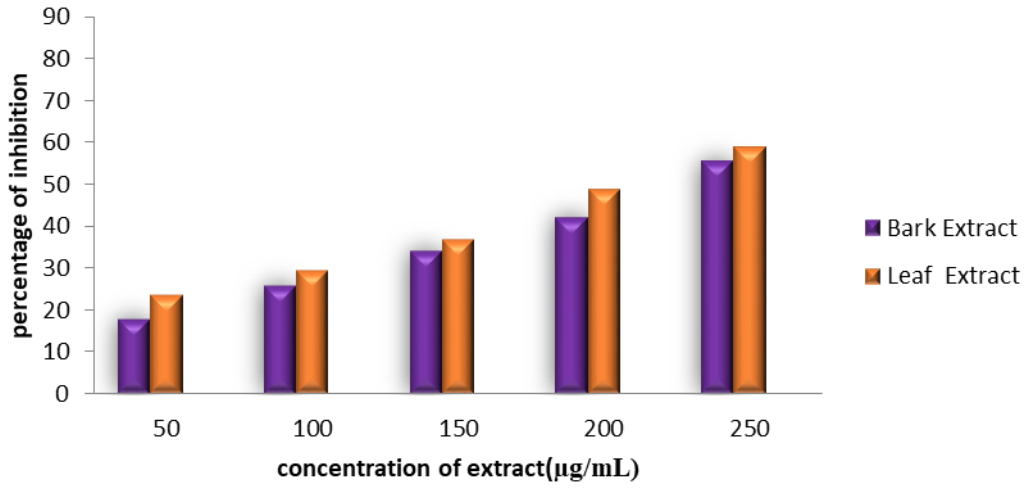


Fig. 4. HRBC membrane stabilization activity of bark and leaf extracts of *Polyalthia longifolia*

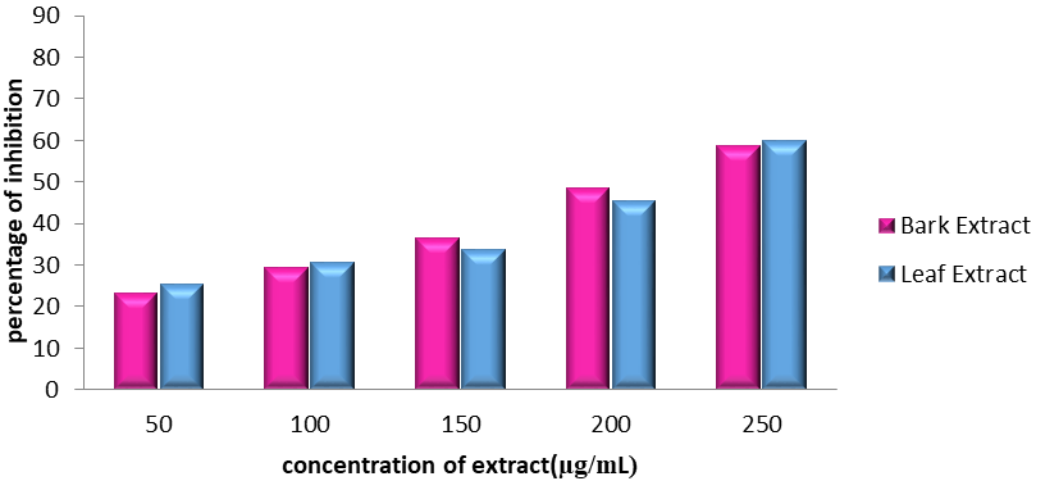


Fig. 5. HRBC membrane stabilization activity of bark and leaf extracts of *Solanum torvum*

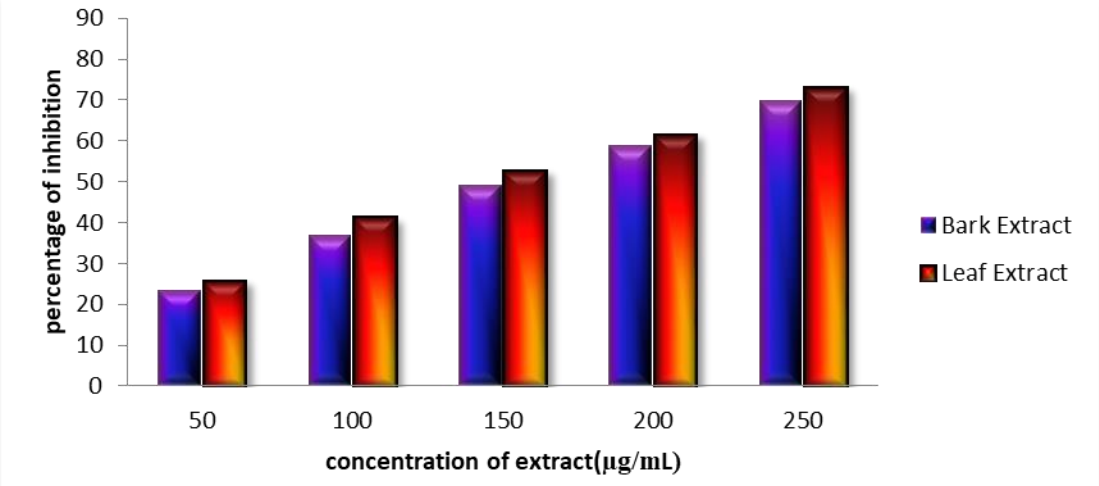


Fig. 6. HRBC membrane stabilization activity of bark and leaf extracts of *Thespesia populnea*

Table 1 α-amylase inhibition activity of *Polyalthia longifolia*

S.No	Conc. of Extract (µg/ml)	Absorbance at 540nm (bark)	% of inhibition	IC_{50} (µg)	Absorbance at 540nm (leaf)	% of inhibition	IC_{50} (µg)
1	control	0.573	-		0.573	-	
2	50	0.435	24.08		0.485	15.35	
3	100	0.290	49.38	105.0	0.427	25.47	187.5
4	150	0.226	60.55		0.231	59.68	
5	200	0.118	79.40		0.142	75.28	

Table 2 α-amylase inhibition activity of *Solanum torvum*

S.No	Conc. of Extract (µg/ml)	Absorbance at 540nm (bark)	% of inhibition	IC_{50} (µg)	Absorbance at 540nm (leaf)	% of inhibition	IC_{50} (µg)
1	Control	0.573	-		0.573	-	
2	50	0.505	11.87		0.497	13.26	
3	100	0.462	19.37	212.5	0.445	22.33	170.0
4	150	0.377	34.21		0.304	46.95	
5	200	0.303	47.12		0.266	53.58	

Table 3 α-amylase inhibition activity of *Thespesia populnea*

S.No	Conc. of Extract (µg/ml)	Absorbance at 540nm (bark)	% of inhibition	IC_{50} (µg)	Absorbance at 540nm (leaf)	% of inhibition	IC_{50} (µg)
1	Control	0.573	-		0.573	-	
2	50	0.516	09.95		0.510	10.99	
3	100	0.487	15.01	222.5	0.462	19.37	210.0
4	150	0.405	29.32		0.392	31.59	
5	200	0.322	43.80		0.306	46.60	

Table 4 HRBC membrane stabilization activity of *Polyalthia longifolia*

S.No	Conc. of Extract (µg/ml)	**Absorbance** at 560nm (bark)	**%** of inhibition	IC_{50} (µg)	Absorbance at 560nm (leaf)	% of inhibition	IC_{50} (µg)
1	control	0.945	-		0.945	-	
2	50	0.779	17.57		0.723	23.49	
3	100	0.702	25.71	282.5	0.667	29.42	207.5
4	150	0.625	33.86		0.598	36.72	
5	200	0.548	42.01		0.485	48.68	
6	250	0.423	55.24		0.389	58.84	

Table 5 HRBC membrane stabilization activity of *Solanum torvum*

S.No	Conc. of Extract (µg/ml)	**Absorbance** at 560nm (bark)	**%** of inhibition	IC_{50} (µg)	Absorbance at 560nm (leaf)	**%** of inhibition	IC_{50} (µg)
1	control	0.945	-		0.945	-	
2	50	0.756	20.00		0.704	25.50	
3	100	0.686	27.40	217.5	0.655	30.69	212.5
4	150	0.647	31.53		0.624	33.97	
5	200	0.523	44.66		0.515	45.50	
6	250	0.402	57.46		0.376	60.21	

Table 6 HRBC membrane stabilization activity of *Thespesia populnea*

S.No	Conc. of Extract (µg/ml)	**Absorbance** at 560nm (bark)	**%** of inhibition	IC_{50} (µg)	Absorbance at 560nm (leaf)	**%** of inhibition	IC_{50} (µg)
1	control	0.945	-		0.945	-	
2	50	0.723	23.49		0.703	25.61	
3	100	0.598	36.72	155.0	0.556	41.16	137.5
4	150	0.480	49.20		0.448	52.59	
5	200	0.390	58.73		0.365	61.38	
6	250	0.285	69.84		0.255	73.02	

PLATE-1

***Polyalthia longifolia*- Habit**

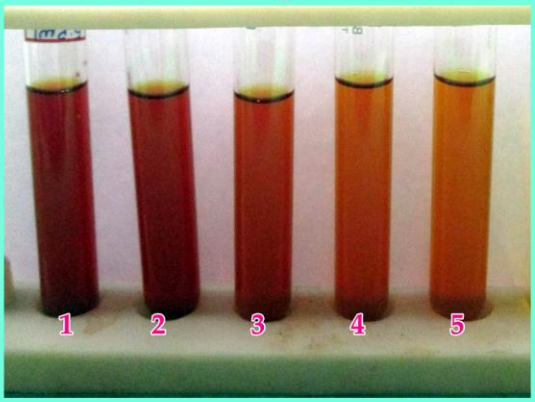
***Solanum torvum* - Habit**

***Thespesia populnea* - Habit**

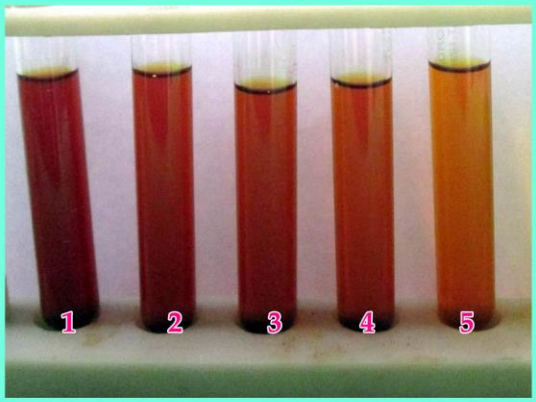
PLATE - 2

Pictures showing Antidiabetic Activity (α - Amylase Inhibitory Assay) of Aqueous Extracts of Selected Medicinal plants

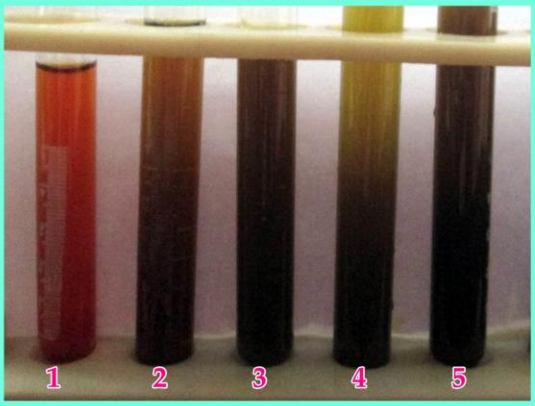
Polyalthia longifolia Bark



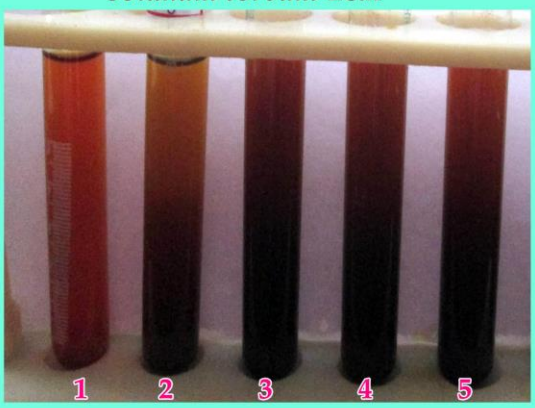
Polyalthia longifolia Leaf



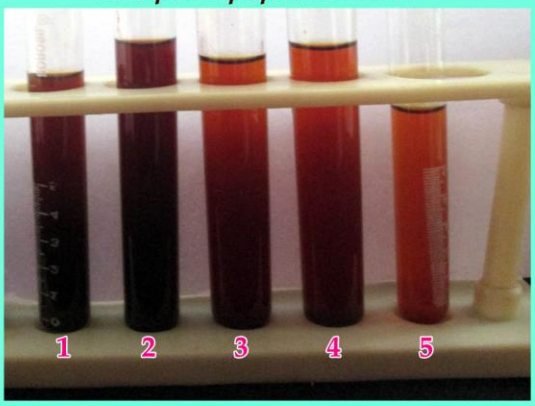
Solanum torvum Bark



Solanum torvum Leaf



Thespesia populnea Bark



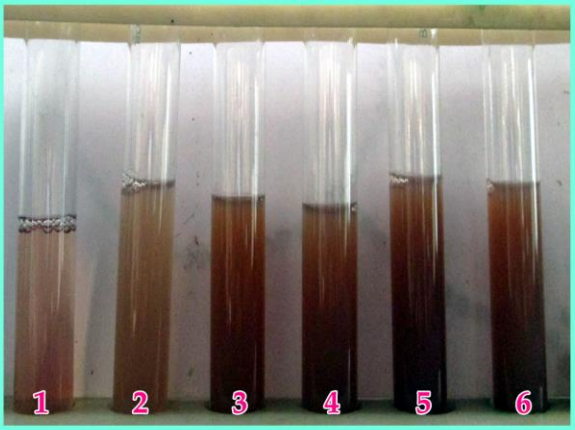
Thespesia populnea Leaf

1- Control 2 - 50 µg 3 - 100 µg 4 - 150 µg 5 - 200 µg

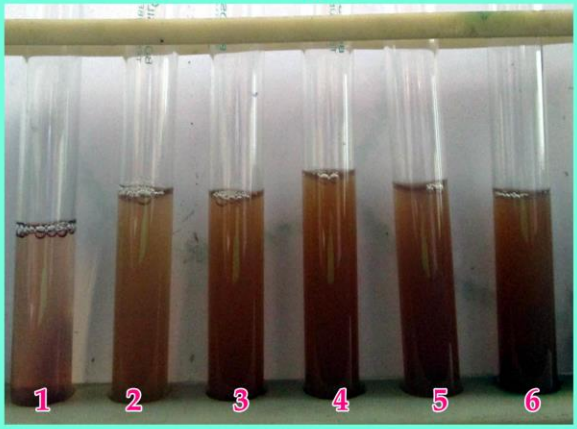
PLATE - 3

Pictures showing Anti-inflammatory Activity of Selected Medicinal plants

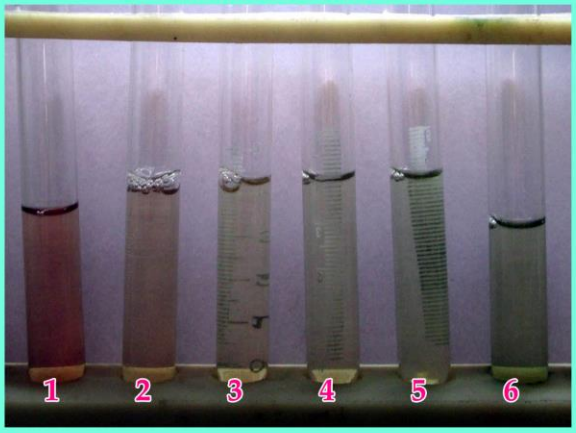
Polyalthia longifolia Bark



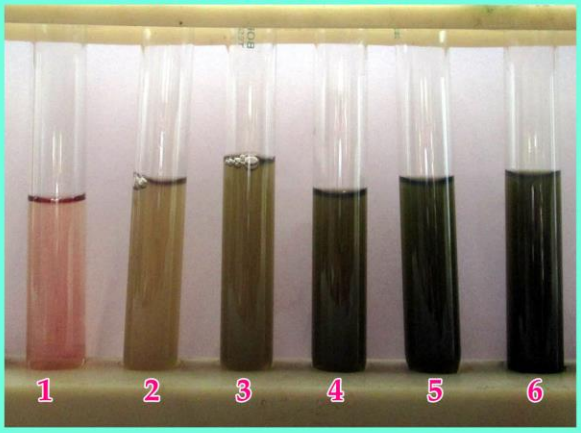
Polyalthia longifolia Leaf



Solanum torvum Bark



Solanum torvum Leaf



Thespesia populnea Bark

Thespesia populnea Leaf

1 - Control
2 - 50 µg
3 - 100 µg
4 - 150 µg
5 - 200 µg
6 - 250 µg

The outcomes of this study show that these plant species may possibly control the postprandial blood glucose ranges and confirm the use of the decoction of herbs suggested as a treatment of diabetes in traditional medicine in many areas. The results of the *in vitro* studies of this investigation indicated that plant species, especially barks of *Polyalthia longifolia* can function as natural amylase inhibitors and might possess beneficial antidiabetic property in the type II diabetes mellitus.

3.2. Membrane stabilization assay

To assess the anti-inflammatory effect, five concentrations of extracts were used. These extracts caused a significant decrease in the lysis of RBC during the experiment. The effects of the different extracts of all the three plants on heat induced hemolysis are summarized in the tables (Tables 4, 5 & 6).

The percentage of inhibition was found to be 58.84% with the leaf extract of *Polyalthia longifolia* and 60.21% with the leaf extract of *Solanum torvum*. *Thespesia populnea* showed highest activity (73.02%) among the three plants studied (Figures 4, 5 & 6). Leaf extract showed more activity than the bark. So it is evident that all the plants used in this experiment possess the compounds which give resistance for the RBC membrane against heat induced lysis (Plate – 3).

The IC_{50} values suggest that the leaf extract of *Thespesia populnea* was more effective in inhibiting HRBC hemolysis (IC_{50} was 137.5 µg). *Polyalthia longifolia* bark showed the minimum activity with an IC_{50} value of 282.5µg. Compounds with membrane stabilizing properties are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators like prostaglandins and leukotrienes *etc.* through COX (cyclooxygenase) and LOX (lipoxygenase) pathway. Membrane stabilisers are chemical compounds which protect erythrocytes against hemolysis and stabilize the lysosomes and cell organelles under *in vitro* conditions [17].

It is believed that compounds with membrane stabilizing properties offer significant protection of cell membrane. In the study of membrane stabilization by hemolytic method, the leaf extract of *Thespesia populnea* found to protect significantly the erythrocyte membrane against lysis induced by heat.

Generally it is believed that tannins, flavonoids and other chemical compounds present in the plant are speculated to account for the observed hypoglycaemic and anti inflammatory effects of the plant extracts. In the present study, the *in vitro* anti inflammatory activity of plant extracts can be attributed to the secondary metabolites present in these plants. Some authors contribute that the effect may be due to the synergistic effect rather than single constituent [18]. Further studies are required to elucidate whether *in vitro* effects represent therapeutic potential of the bioactive compounds.

4. CONCLUSION

The aqueous extracts of leaf and bark of three different plants were tested for α-amylase inhibition activity and erythrocytes membrane stabilization ability. For the analysis of α- amylase inhibitory activity DNSA method was followed. Different concentrations of extracts were incubated with enzyme substrate solution and the activity of enzyme was measured. HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by heat induced membrane lysis was taken as an *in vitro* measure of anti inflammatory activity. The bark extract of *Polyalthia longifolia* was found to be more potent than the other extracts analyzed which showed the highest percentage of inhibition of α-amylase (79.40%) at the concentration of 200 µg. The leaf extract of *Thespesia populnea* showed highest

inhibitory activity (73.02%) over hemolysis among the three plants studied. Standard procedures were followed in the *in vitro* studies, which are validating the traditional claim of the plants that they possess medicinal properties. Further research is needed in future to identify the phytochemical constituents responsible for such activities and their exploration in the pharmaceutical industries.

ACKNOWLEDGEMENTS

The authors acknowledge the infrastructural support received from the department of Botany, Holy Cross College (Autonomous), Tiruchirappalli, for their support and encouragement in carrying out this work.

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