The Journal of Phytopharmacology 2020; 9(5): 356-360
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2020; 9(5): 356-360
September- October
Received: 20-08-2020
Accepted: 25-09-2020

doi: 10.31254/phyto.2020.9511

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In vitro anti-inflammatory activity of aqueous extract of *Albizia lebbeck* leaf (l.)

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ABSTRACT

Albizia lebbeck (L.) Benth is an important traditional tree found throughout India. All part of this tree is considered as folk medicine and used for treatment of various disease. Current research work was carried out to identify the bioactive compound by phytochemical screening and to evaluate anti-inflammatory potential of aqueous leaf extract of *A. lebbeck*. The phytochemical screening of the leaf of *A. lebbeck* shows the presence of alkaloid, flavonoid, tannin, phenol, saponin, glycoside and free amino acid. The maximum inhibition of protein denaturation was found to be 78.06±0.5% at 500 µg/mL concentration and its IC_{50} was 330 µg/mL concentration. The maximum inhibition in membrane stabilization was found to be 74.09±0.33% at 500 µg/mL concentration and its IC_{50} was 440 µg/mL concentration. The maximum protection in hypotonicity induced haemolysis shows about 69.34±0.38% at 500 µg/mL concentration and its IC_{50} was 400 µg/mL concentration. *In vitro* assay shows the moderate activity of anti-inflammatory in aqueous extract of *A. lebbeck*, when compared with the standard.

Keywords: *Albizia lebbeck*, Anti-inflammatory, Denaturation, Membrane stabilization.

INTRODUCTION

Inflammation is the local response in living tissue of mammal caused by injury or infection due to causative agent such as physical, chemical, infective and immunological agent. It is one of innate defense mechanism in the body. Inflammation is an important for immune response by the host which is enabled by the removal of harmful stimuli along with healing of damaged tissue. In higher organisms it plays a protective role in the host for renewing cellular homeostasis in injured or damage condition [1]. Anti-inflammatory drug which are available currently in market have serious side effects, relief from symptoms and block enzyme activity. Research works focus on the medicinal plants for lesser side effects in the formulation of drug [18, 19].

Medicinal plants found to be an essential for natural wealth of a country. It acts as therapeutic agent and serves as an important raw material for manufacture of traditional and modern medicine [10]. Bioactive compound derived from the medicinal plants namely phytochemical which has role in pharmacological activity [17]. Various bioactive compounds are screened from plants which leads for the development of new medicinal drug, responsible for systematic protection and treatment of various diseases [14]. *Albizia lebbeck* is one of the traditional species of *Albizia* present in worldwide (Figure 1). It is widely cultivated in tropical and subtropical region as ornamental plant. Lot of data are present on therapeutic property. All part of this tree is well-known for traditional folk medicine. Genus *Albizia* is rich in bioactive compounds which involve Secondary metabolite such as saponin, tannin, alkaloids are present [2].

Figure 1: Leaves and seed pod of *Albizia lebbeck*

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The medicinal property of *Albizia lebbeck* tree includes antiseptic, anti-microbial, anti-fertility, anti-protozoal, anti-dysentric, anti-tubercular, anti-cancer, nootropic, anxiolytic, anti-convulsant [4, 15].

MATERIAL AND METHOD

Sample collection and condensation process

The leaf of *A. lebbeck* was collected from the place of Chinnadharapuram, Karur, Tamil Nadu, India. Plant material is authenticated by Botanical survey of India, Southern Regional Centre, Coimbatore (BSI/SRC/5/23/2019/Tech-262). The leaf was washed with tap water and kept for shade dry. Then the leaf is made into coarse of powder and immersed in aqueous for 48 hours. The plant residue is filtered and condensed by using the process of lyophilization which yield crystalline powder.

Qualitative phytochemical analysis

In Systematic study of crude drug, a detailed profile of both primary and secondary metabolite is required. The phytochemical constituents present in plants plays a major role in the field of pharmaceutical because it is needed for the formulation of new drug. The aqueous extract of *A. lebbeck* was subjected for preliminary phytochemical screening using specific standard method [5, 8, 16].

In Vitro Anti-Inflammatory Activity

Inhibition of Protein denaturation

Denaturation of protein occurs as any change that alters the unique 3D conformation of a protein molecule resulting in cleavage of peptide bond. Protein denaturation has been associated with the formation of inflammatory disorders like rheumatoid arthritis, diabetes and cancer. Prevention of protein denaturation help to save for inflammatory disorders [12].

Inhibition of protein denaturation can be studied by using egg albumin (or) BSA as a protein. The anti-inflammatory activity of aqueous leaf extract of *A. lebbeck* was studied by using inhibition of protein denaturation assay which was studied according to Mizushima *et al* & Sakat *et al* with slight modification [9, 11]. The reaction mixture consists of 1ml of sample with various concentration (100 -500µg/mL) and 1% aqueous solution of bovine albumin fraction pH 6.5 of which the reaction is adjusted using small amount of 1N HCl. Diclofenac sodium was used as a standard drug. All the reaction mixture was incubated at 37 °C for 20 minutes and heated at 70°C for 5 mins. After cooling the samples, the turbidity was measured Spectrophotometrically at 660nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as

Percentage Inhibition = (s control – Abs sample) x 100/Abs control.

Membrane Stabilization

For understanding the mechanism of anti-inflammatory activity, Inhibition of heat induced lysis and hypotonicity of RBC membrane was taken because Human RBC is similar to lysosomal membrane. The determination of membrane stabilizing activity of the extract is done through heat induced haemolysis and hypotonic solution induced haemolysis through heat induced haemolysis through human erythrocytes. Non-steroidal anti-inflammatory drugs (NSAIDS) show their effects where by the inhibition of lysosomal enzyme (or) lysosomal membrane gets stabilized. Lysosomal membrane gets lysed during inflammation where the enzyme component occurs. Inflammatory response is controlled by the lysosomal membrane stabilization [6, 13].

Preparation of Red Blood Cells (Rbc) Suspension

The blood was collected from healthy volunteers and transfer to the centrifuge tubes. The tubes were centrifuged at 3000rpm for 10 min. The pellet was washed three times with equal volumes of normal saline. Measure the volume of blood and reconstituted as 10%v/v Suspension with normal saline [7].

Heat Induced Haemolysis

The reaction mixture contains 2ml of the sample with various concentration (100-500 µg/mL) and 1ml of 10% RBCs suspension. For the control tube 1ml of saline is added instead of sample. Aspirin is used as standard reference. All the tubes were incubated in water bath at 56°C for 30 mins. After incubation the tubes are cooled and centrifuged at 2500rpm for 5 mins and the supernatant were taken at the absorbance at 560nm. The experiment is done in triplicates for all test sample [3].

The percentage inhibition of haemolysis was calculated as

Percentage Inhibition = (Abs control – Abs sample) x 100/Abs control.

Hypotonicity Induced Haemolysis

The reaction mixture contains Control, standard drug and various concentration of extract (100-500µg/mL) with 1ml of phosphate buffer in separate test tube. 2ml of hyposaline and 0.5ml of HRBC suspension are added to all the test tube. This reaction is incubated at 37°C for 30 min and centrifuged at 3000rpm. The supernatant liquid was decanted and the amount of haemoglobin was estimated at 560nm [3]. Aspirin is used as standard reference. The percentage protection was calculated as

Percentage Protection = 100-(OD Sample / OD Control) x 100.

RESULTS AND DISCUSSION

Phytochemical Screening

The results for the preliminary phytochemical screening of Aqueous extract of *A. lebbeck* was shown in the Table 1. Thereby, it was observed the presence of alkaloid, flavonoid, phenol, saponin, glycoside and free amino acid.

Table 1: Phytochemical screening of Aqueous extract of *A. lebbeck*

S.NO	Phytochemicals	Test	Result
1	Alkaloid	Dragendroff reagent	+
2	Flavonoid	Lead acetate test	+
3	Phenol	Ferric chloride test	+
4	Saponin	Froth test	+
5	Glycoside	Bontrager test	+
6	Free amino acid	Ninhydrin test	+

In methanol extract of *Enicostemma axillare* the activity of anti-inflammatory may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids and phenol [7].

***In Vitro* Anti-Inflammatory Activity**

Inhibition of Protein denaturation

Denaturation of protein is well documented for cause of inflammation. In heat induced albumin denaturation inhibition is very effective. The mechanism of the anti-inflammatory activity investigates the ability of plant extract by the inhibition of protein denaturation assay. The inhibitory effect of the sample was shown in the Table 2. The maximum inhibition of protein denaturation for the sample and standard (Diclofenac sodium) at 500 µg/mL was found to be 78.06% and 81.14% respectively. IC_{50} value for the sample and the standard was observed as 330 µg/mL and 360 µg/mL respectively. From the above results, it was identified that the sample is having less protein denaturation.

Table 2: Inhibition of protein denaturation assay of aqueous leaf extract of *A. lebbeck*

S.NO	Concentration (µg/mL)	% Inhibition of standard *	% Inhibition of sample*
1	100	39.90±0.26	37.11±0.38
2	200	48.26±0.46	46.20±0.53
3	300	57.85±0.54	52.79±0.25
4	400	67.05±0.65	63.90±0.48
5	500	81.14±0.44	78.06±0.51

(*Average value of 3 replicates)

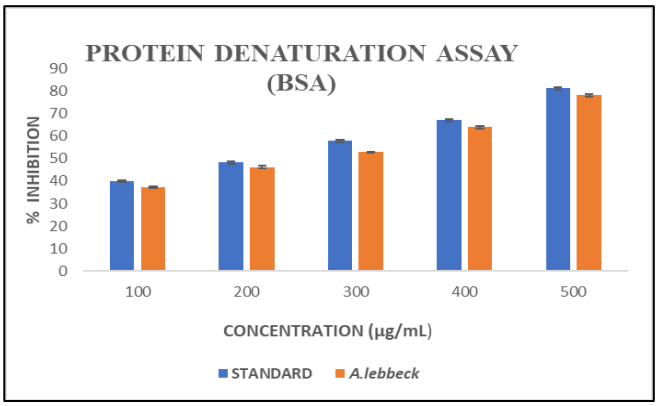

Figure 2: Inhibition of protein denaturation assay of aqueous leaf extract of *A. lebbeck*

Denaturation of protein is a biochemical reaction which take place during chronic inflammatory response resulting in loss of function in tissue. Methanol extract of *Enicostemma axillare* showed the higher inhibition of protein denaturation when compared to standard drug [7].

Membrane Stabilization

Heat Induced Haemolysis

Human erythrocytes are used for the study of *in vitro* anti-inflammatory activity by the method of HRBC Membrane stabilization. Investigation on HRBC membrane stabilization is done because it is similar to lysosomal membrane. Due to the similar of human erythrocytes as in lysosomal membrane, the possible effect of aqueous extract of *A. lebbeck* may inhibit the release of lysosomal content in neutrophils during inflammation. In heat induce haemolysis of aqueous leaf extract of *A. lebbeck* shows significant protection at 74.09% at 500 µg/mL and IC_{50} 440 µg/mL (Table 3 and Figure 3). Aspirin is used as standard drug where the maximum inhibition is 91.05% at 500 µg/mL and IC_{50} µg/mL.

Table 3: Heat induced haemolysis of aqueous leaf extract of *A. lebbeck*

S.NO	Concentration (µg/mL)	% Inhibition of standard *	% Inhibition of sample*
1	100	62.90±0.34	45.19±0.37
2	200	85.23±0.33	64.35±0.45
3	300	87.59±0.36	67.83±0.48
4	400	89.64±0.48	71.83±0.43
5	500	91.05±0.38	74.09±0.33

(*Average value of 3 replicates)

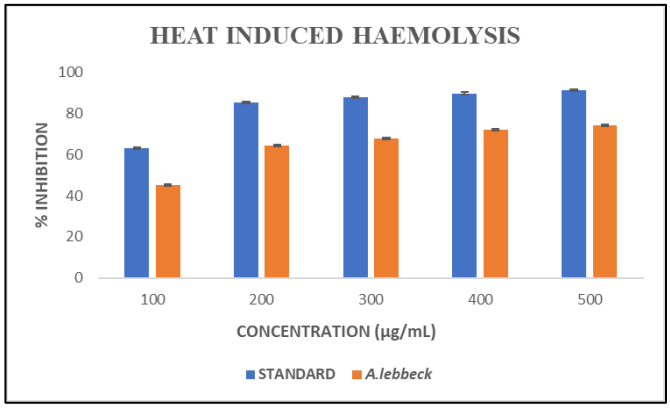

Figure 3: Heat induced haemolysis of aqueous leaf extract of *A. lebbeck*

In lysosomal stabilization, the limiting of inflammatory response is important. After limiting the inflammatory response, the release of lysosomal constituted are prevented. Methanol extract of *Enicostemma axillare* showed significant protection in erythrocytes membrane against heat induce haemolysis [7].

Hypotoncity Induced Haemolysis

The phenomenon based on protective effect in hypotonic saline induced in erythrocyte is well known and a good for anti-inflammatory activity. The significant protection shows at 69.34% at 500 µg/mL concentration in aqueous extract of *A. lebbeck* and IC_{50} is 400 µg/mL concentration (Table 4 and Figure 4). Aspirin is used as standard drug. It shows high significant protection in 85.80% at 500 µg/mL concentration and IC_{50} is 410 µg/mL concentration.

Table 4: Hypotonicity induced haemolysis of aqueous extract of *A. lebbeck*

S.NO	Concentration (µg/mL)	% Inhibition of standard *	% Inhibition of sample*
1	100	36.75±0.55	16.50±0.46
2	200	48.99±0.27	28.00±0.54
3	300	69.41±0.45	48.94±0.52
4	400	71.98±0.46	57.74±0.45
5	500	85.20±0.36	69.34±0.38

(*Average value of 3 replicates)

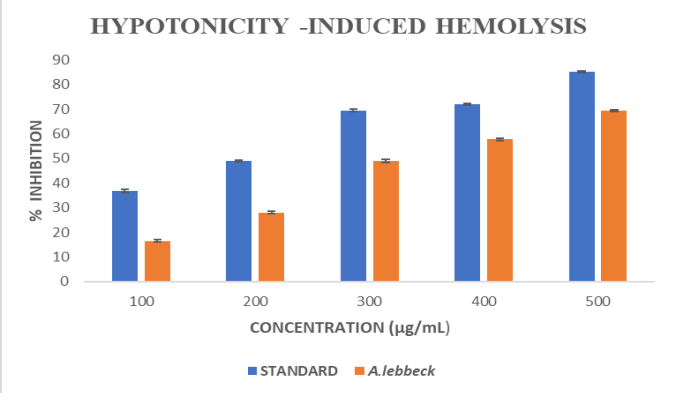

Figure 4: Hypotonicity induced haemolysis of aqueous leaf extract of *A. lebbeck*

Methanol extract of *Enicostemma axillare* shows significant protection while comparing with diclofenac the standard drug for anti-inflammation [7].

CONCLUSION

Phytochemical compound found in aqueous leaf extract of *A. lebbeck* shows a potential for the formulation of anti-inflammation drugs. *In vitro* assay for anti- inflammatory activity of aqueous leaf extract of *A. lebbeck* studies provides the moderate activity. The present study illustrates that the aqueous extract of A. *lebbeck* has significant for anti-inflammation activity. Hence, the sample can be suggested for the formulation of anti- inflammatory drug. The existing work provides an idea that the sample can be used for discovering a new anti-inflammatory drug. Isolation, Purification and Characterization of the sample may be used for the future research.

Acknowledgment

The author is thankful to Department of Biochemistry PSG College of Arts & Science, Coimbatore for providing required facilities to carry out this analytical work. I am also thankful to Dr. S. Valarmathi, Assistant Professor, Department of Biochemistry, PSG College of Arts & Science, for guiding me throughout this work.

Conflict of interest

The authors declare no conflict of interests

Funding

No funding was received for this study.

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HOW TO CITE THIS ARTICLE
Kamala Lakshmi B, Valarmathi S. *In vitro* anti-inflammatory activity of aqueous extract of *Albizia lebbeck* leaf (l.). J Phytopharmacol 2020; 9(5):356-360.