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IN-VITRO EVALUATION OF ANTIARTHRITIC ACTIVITY OF *LAGERSTROEMIA SPECIOSA* PLANT EXTRACT

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ABSTRACT

Aim: This study aims to evaluate the antiarthritic activity of aqueous and ethanolic extracts of *Lagerstroemia specicosa*. **Materials and Methods:** Plant was collected and then powdered; the powdered plant was then cold macerated with 70% v/v of ethanol and Aqueous for 48 h and 72 h, respectively. Both extracts, after the subsequent time, were concentrated to a syrupy mass in Soxhlet apparatus under reduced pressure and desiccated. Protein denaturation assay is based on the effect of plant extract to inhibit the happening of denaturation of protein from heat. We evaluated the potency of the plant extract to encumber the denaturation of protein. It was calculated in percentage and absorbance was studied at 660 nm. **Results and Discussion:** On increasing concentrations, it exhibited different percentage inhibition. Among aqueous and ethanol extract, ethanolic extract was found to be equally effective when compared to standard drug diclofenac sodium. In antidenaturation assay, the effect of plant extract was determined on the basis of its potential to inhibit the denaturation of the protein. **Conclusion:** Study indicates that the plant extract has the potency to exhibit antiarthritic activity and can be used to proceed with further *in vivo* studies and also the isolation of principle constituent.

KEYWORDS: Denaturation percentage inhibition, Soxhlet, Anti-arthritic activity; *L. speciosa.*

INTRODUCTION

Lagerstroemia specicosa L. Pers, Lythraceae, have been used in traditional medicine to treat diabetes mellitus in Southeast Asia for a many years. This tree is considered to be sacred in many places in India. L. speciosa leaves with aqueous acetone led to the isolation of seven ellagitannins, ellagic acid, ellagic acid sulphate and four methyl ellagic acid derivatives, including corosolic acid, gallic acid, 4-hydroxybenzoic acid, 3- O-methyl protocatechuic acid, caffeic acid, p-coumaric acid, kaempferol, quercetin and isoquercitrin. L. speciosa leaves are consumed as herbal tea for lowering blood sugar level and reducing body weight, while in India, they are used as a remedy for diabetes. The edible flowers are used for garnishing dishes or as ingredients in salads, soups, desserts and drinks. In recent years, herbal products such as Banabamin and Glucosol TM have been developed from L. specicosa for use as anti-diabetic drugs, with preliminary clinical trials conducted. From the aqueous ethanol leaf extract of L. speciosa, six new monomeric and dimeric ellagitannins (flosin A and B, and reginin A, B, C and D), and three new ellagitannins (lagerstannins A, B and C) were isolated and identified. Further extraction of L. specicosa leaves with aqueous acetone led to the isolation of seven ellagitannins, ellagic acid, ellagic acid sulphate and four methyl ellagic acid derivatives, including corosolic acid, gallic acid, 4-hydroxybenzoic acid, 3- O-methyl protocatechuic acid, caffeic acid, p-coumaric acid, kaempferol, quercetin and isoquercitrin. The whole plant extract has been reported to possess potent anti-inflammatory, antibacterial, and antioxidant and several activities.

In this study, we are performing denaturation assay for the evaluation of arthritic activity of the plant using Aqueous and ethanolic extracts. During the assay, the extract with increasing concentrations exhibited increasing activity. Maximum concentration of samples and standard showed maximum response, where ethanolic extract was relatively close to standard.

MATERIALS AND METHODS

Collection and Processing of the Plant

The Fresh leafs of lagerstroemiea specicosa were collected from chinar park, M.p. nagar, Bhopal, Madhya Pradesh, in the month of May-June. The plant was authenticated by Dr. Zia ul. Hasan, HOD, Department of Botany, Safia college of Bhopal, Madhya Pradesh (India).The collected plant was free from diseases and also free from contamination of other plants. Before extraction, the plants were air dried for few days and then pulverized. The obtained powder was used for extraction procedures using different solvents.

Preparation of Extract

2 kg of the powdered was cold macerated with 70% v/v of ethanol and aqueous for 48 h and 72 h, respectively. Both extracts, after the subsequent time, were concentrated to a syrupy mass in Soxhlet apparatus under reduced pressure. Then later, it was stored in a silica desiccator. After desiccation, 7% w/w yield was obtained.

Antidenaturation Assay

Protein denaturation assay is based on the effect of plant extract to inhibit the happening of denaturation of protein from heat.

Principle

In this study, it is the measure of an individual plant extract to inhibit the denaturation that is possessed by heat. Whenever the heat is exposed to protein, they generally start to get degraded and collapsed. The aim of this study is to evaluate the potency of the plant extract to encumber the denaturation of protein. It was calculated in percentage and absorbance was studied at 660 nm.

Reagents Required

- Dimethylformamide (DMF).
- Phosphate buffer (0.2 M, PH 7.4).
- 1 ml of 1 mM albumin solution.
- Preparation of extracts (10, 50, 100, 200, 400, 800, and 1000 µg/ml) in DMF.
- Test solution 4 ml.

Procedure

The experiment was carried out with minor modification. The standard drug and extract was dissolved in minimum quantity of DMF and diluted with phosphate buffer (0.2 M, PH 7.4). Final concentration of DMF in all solution was <2.5%. Different concentrations of test solution of drug were mixed with the albumin solution earlier prepared in phosphate buffer and incubated for 15 min in incubator at 37°C. Denaturation was induced by keeping the reaction mixture at 70°C in water bath for 15 min. After cooling, the turbidity was measured at 660 nm. Using the control value, the percentage of inhibition was calculated. The diclofenac sodium was used as standard drug.

The percentage inhibition of denaturation was calculated using following formula:[7]

% of inhibition = 100 × (At-Ac)/At At(S) = O.D. of testsolution.
Ac(C) = O.D. of control.

RESULTS AND DISCUSSION

Data Analysis

In this assay, we can clearly see that increase in concentration is equal to the increase in the potency. Among the concentrations used, 100 µg/ml was found to be more effective and was relatively equal to the standard that has been used. Percentage inhibition of the standard was found to be 79.44, but the ethanolic extract has showed 53.83 which is very much close. Whereas, the aqueous extract exhibited 39.38 as its percentage inhibition at the maximum concentration.

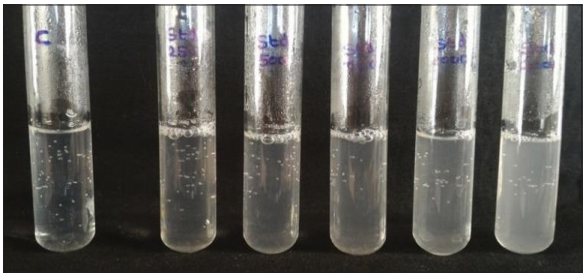


Figure 1: Testing samples of standard.

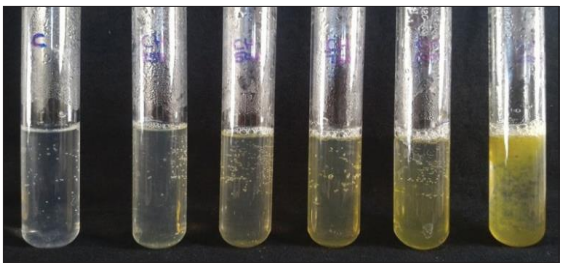


Figure 2: Testing samples of Aqueous extract.

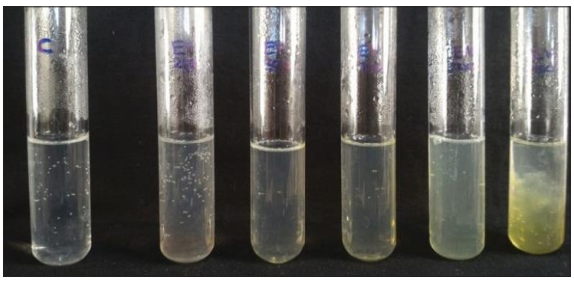


Figure 3: Testing samples of ethanolic extract.

On increasing concentrations, it exhibited different percentage inhibition. Among aqueous and ethanol extract, ethanolic extract was found to be equally effective when compared to standard drug diclofenac sodium. In antidenaturation assay, the effect of plant extract was determined on the basis of its potential to inhibit the denaturation of the protein.

Denaturation was induced with the help of heat at 70°C in water bath for 15 min. Albumin was considered as a protein and with the effect of our extract to prevent the protein from heat was measured. It was read under 660 nm and calculated using appropriate formula. Percentage of inhibition of denaturation was calculated from control where no drug was added.

Tables 1-3 summarize the values that were obtained during the study with respect to the different concentrations and were tabulated accordingly. In Table 1, values of ethanolic extract were entered and it shows the best percentage for the inhibition when compared to the standard. Similarly, in Table 2, Aqueous extract values were tabulated and it also shows an equally good response but not close enough to ethanolic extract. Both of them were compared with the standard drug in Table 3.

Table 1: Percentage inhibition of ethanolic extract of *L.specicosa.*

Sample	Concentration	Absorbance	% inhibition
	20	0.119	25.2605042
	40	0.126	30.96825397
Ethanol	60	0.157	34.05732484
	80	0.252	44.98412698
	100	0.311	53.83048232
	Control	0.058	

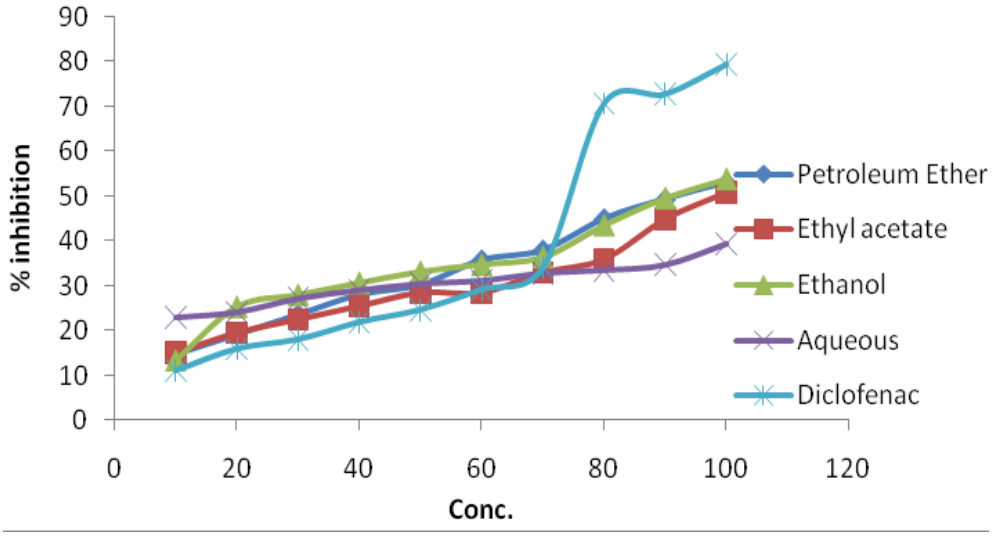
Table 2: Percentage inhibition of Aqueous extract of *L.stroemia.*

Sample	Concentration	Absorbance	% inhibition
	20	0.068	19.70588235
	40	0.085	22.76470588
Aqueous	60	0.108	27.2962963
	80	0.135	33.03703704
	100	0.205	39.38731707
	Control	0.058	

Table 3: Percentage inhibition of diclofenac sodium.

Sample	Concentration	Absorbance	% inhibition
	20	0.141	58.86524823
	40	0.193	62.94818653
Diclofenac	60	0.289	67.93079585
Sodium	80	0.348	70.33333333
	100	0.476	79.44512605
	Control	0.058	

Graph 1 was also incorporated using the values to indicate the rise in activity with respect to the increase in concentration and Figures 1-3 show the samples before reading the absorbance.



CONCLUSION

Our investigation has clearly demonstrated the potential of the *L. specicosa* plant extract to possess antiarthritic property. It may be due to the presence of chemi- cal components such as Hentriacontane, (4,6,8-Tri-ter-butyl-9-oxa-tricyclo deca-2,4,6-trien-1-yl)-methanol. However, further, the aim of the study is to perform *in vivo* studies to authenticate the biological activity.

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