Investigation of Anti-arthritic Activity of *Anthocephalus candamba* Roxb. (Ma U) Bark

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Abstract

The *in vitro* anti-arthritis activity of watery and ethanol extracts of *Anthocephalus cadamba* Roxb. (Ma u) bark was determined by protein denaturation method. The maximum anti-arthritis activity was observed in the concentration of 500 µg / mL while the minimum anti-arthritis activity was observed in the concentration of 125 µg / mL. The percentage of arthritis protection was found to be 45.58 % in ethanol extract and 31.62 % in watery extract at the concentration of 500 µg / mL in bovine serum albumin denaturation method. In egg albumin denaturation method was found to be 62.5 % in ethanol extract and 30.55 % in watery extracts at the concentration 500 µg / mL. Inhibition of joints inflammatory was observed in concentration 125 µg / mL for each extracts.

Keywords: *Anthocephalus cadamba* Roxb., anti-arthritis activity, bovine serum albumin, egg albumin

Introduction

Anthocephalus cadamba Roxb. (Ma u) is one of the important medicinal plants in India, belonging to the Rubiaceae family. *A. cadamba* Roxb. is commonly known as *Cadamba* and *Kadamba* (Sanskrit and Hindi), Bur-flower tree (England), sako (Laos), laran (Malaysia) and ma u or ye ma u (Myanmar). It is an ever green tree found in different parts of India, Nepal, Laos, Cambodia, China, Malaysia and Myanmar. The bark is gray, smooth in young trees and rough in old trees. Leaves are glossy green, opposite, simple more or less sessile to petiole, ovate to elliptical (15-50 × 8-25 cm). Inflorescences are clusters, fragrant, orange or yellow flowers. It grows best on deep, moist, alluvial sites, tropical climate, permanently flooded area, periodically flooded area. It is found in India, Nepal, Laos, Cambodia, China, Malaysia and Myanmar (Dubey, *etal*., 2011). *A.cadamba* Roxb. can cure several diseases and ailments such as diabetes, cardiovascular disorders, cancer and liver damage. A variety of plants is used for medicinal treatments; either whole or in specific parts (bark, root, leaves, fruit, flowers and seeds) in the dried state (Vartak, *etal*., 1987).The bark of the *A.cadamba* Roxb. is used to anti-inflammatory, digestive, and diuretic property and is given to treat and inflammation of eyes (Ram and Liza 2011). Different parts of this plant have been used in several medicinal purposes including the facts that bark is tonic and the leaves are slightly aromatic. Flowers are an important raw material in the production of 'attar' which is Indian perfume with sandalwood base and source of an essential oil. *A.cadamba* Roxb. is crucially significant as it has the largest number of phytochemicals and secondary metabolites having pharmacological and biological properties. These are consumed using water, sugar, salt, honey, etc. Now, they are formulated into suitable preparations such as tablets, pills, extracts, tinctures, lotions, ointments and cream (Sahu, *etal.*, 1999). This research work was to investigate the anti-arthritis activity.

(a) (b)

Figure 1. (a) Plant and (b) bark of *A. cadamba* Roxb. (Ma u)

Materials and Methods

Sample Collection and Preparation

The barks of *Anthocephalus cadamba* Roxb. (Ma u) were collected from Sein-Kone Village, Pathein Township, Ayeyarwady Region in January, 2018. Then, the sample was identified at the Department of Botany, University of Yangon.

The fresh barks were cleaned by washing with water and air dried at room temperature for two weeks. The dried barks were cut into small pieces and were made into powder by using grinding machine. The dried powdered samples were stored in air tight container to prevent moisture changes and other contaminations. The dried powdered samples were used to investigate for their chemical and biological activities.

Procedure for Anti-arthritis activity

Anti-arthritis activity of crude extracts of *A. cadamba* Roxb. bark was studied using bovine serum albumin denaturation method and egg albumin denaturation method (Ram, *etal*., 2015).

(i) Bovine Serum Albumin (BSA) Denaturation Method

0.05 mL of various concentrations (500, 250, 125 µg / mL) of test samples and standard drug diclofenac sodium (500, 250, 125 µg / mL) were taken respectively and 0.45 mL (0.5 % w/v BSA) mixed. The samples were incubated at 37 °C for 30 minutes and the temperature was increased to keep the samples at 70 °C for 5 minutes. After cooling, 2.5 mL of phosphate buffer was added to the above solutions. The absorbance was measured using UV-visible spectrophotometer at 660 nm. The control represents 100 % protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation was calculated by this formula.

Percent inhibition = × 100

(ii) Egg Albumin Denaturation Method

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations (500, 250, 125 µg / mL) of drug. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at 37 °C in incubator for 30 minutes and then heated at 70 °C for 5 minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium in the concentrations (500, 250, 125 µg / mL) was used as the reference drug and treated similarly for the determination of absorbance.

Results and Discussion

Anti-arthritis activity

Denaturation of tissue protein is one of the known causes of inflammatory and arthritis diseases. Also the production of auto antigens may be due to denaturation of protein. In the present study, protein denaturation and albumin denaturation methods were selected for *in vitro* assessment of anti-arthritis activity of watery and ethanol extracts of bark of *Anthocephalus cadamba* Roxb. (Ma-u). The standard anti-arthritis activity drug; diclofenac sodium was used for these tests. The absorbance of different concentrations (500, 250, 125 µg / mL) of tested samples was measured at 660 nm by using UV-visible spectrophotometer.

The *in vitro* anti-arthritis activity of watery and ethanol extracts of *A. cadamba* Roxb. (Ma u) by protein denaturation method using bovine serum albumin was shown in Table 1 and Figure 1. The water and ethanol extracts of *A. cadamba* Roxb. and Diclofenac sodium were tested at different concentrations for anti-arthritis activity and found significant percentage inhibition in protein denaturation. The maximum anti-arthritis activity was observed in the concentration of 500 µg / mL while the minimum anti-arthritis activity was observed in the concentration of 125 µg / mL. According to result, the percentage of arthritis protection was found to be 45.58 % in ethanol, 31.62 % in watery extracts and 97.79 % in Diclofenac sodium at the concentration of 500 µg / mL in bovine serum albumin denaturation method.

In egg albumin denaturation method was found to be 62.5 % in ethanol, 30.55 % in watery extracts and 97.22 % in Diclofenac sodium at the concentration 500 µg / mL. These results were observed in Table 2 and Figure 2. In both methods, the ethanol extract has shown significant activity at the concentrations of 500 µg / mL and the effects were compared with the standard drug diclofenac sodium. From the present study, ethanol extract of *A. cadamba* Roxb. (Ma u) bark has higher anti-arthritis activity than watery extract.

Table 1. Results of Anti-arthritis Activity of Ethanol and Water Extracts of Bark of *A.cadamba* Roxb. by Protein Denaturation Method Using Bovine Serum Albumin

Extracts	Concentration (μg / mL)	Absorbane at 660 nm	% Inhibition
Control	-	0.068	
Ethanol	125	0.051	16.91
	250	0.040	40.44
	500	0.030	45.59
Water	125	0.052	12.5
	250	0.050	21.32
	500	0.042	31.62
Diclofenac sodium	125	0.010	84.56
	250	0.006	91.17
	500	0.001	97.79

Figure 1. Percent Inhibition of protein denaturation of watery and ethanol extracts of bark of *A. cadamba* Roxb. and standard drug (diclofenac sodium) using bovine serum albumin

Table 2. Results of Anti-Arthritis Activity of Ethanol and Watery Extracts of Bark of *A. cadamba* Roxb. by Protein Denaturation Method Using Egg Albumin

Extracts	Concentration (μg / mL)	Absorbance at 660 nm	% Inhibition
Control	-	0.036	
Ethanol	125	0.019	47.22
	250	0.015	56.94
	500	0.013	62.5
Water	125	0.031	13.88
	250	0.029	18.05
	500	0.025	30.55
Diclofenac sodium	125	0.004	88.88
	250	0.002	93.05
	500	0.001	97.22

Figure 2. Percent Inhibition of protein denaturation of watery and ethanol extracts of bark of *A. cadamba* Roxb. and standard diclofenac sodium by using egg albumin

Conclusion

These findings suggested that both extracts are capable of controlling the production of auto antigen to inhibit the inflammation of joints. Inhibition of joints inflammatory was observed in concentration 125 µg / mL for each extracts. The percent inhibition of protein denaturation of bark of (Ma u) and Standard drug (diclofenac sodium) with respect to control indicated the stabilization of albumin protein. The findings from the present work will contribute to the scientific development of Myanmar traditional medicine, specifically in the areas concerned with arthritis activity.

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