



Research Article

IN VITRO ANTIRHEUMATOID ARTHRITIC ACTIVITY OF AQUEOUS ROOT EXTRACT OF CLITORIA TERNATEA

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ABSTRACT

In ethano medicine, roots of *Clitoria ternatea* (Family: Fabaceae) is claimed to possess antirheumatoid arthritic activity. However, this claim has not been scientifically tested and validated yet. This study assessed the antirheumatoid arthritic potential of an aqueous root extract (ARE) of *Clitoria ternatea* (Family: Fabaceae) using a well recognised *in vitro* bioassay model: inhibition of heat induced denaturation of albumin protein, which is claimed to act as an index of anti arthritic activity. Five concentrations of ARE (31.25, 62.50, 125, 250 and 500 µg/ml) and six concentrations of reference drug, Diclofenac sodium (78, 125, 156.25, 312.50, 625, 1250 and 2500 µg/ml), were used in the testing. The results showed, for the first time, that ARE of *Clitoria ternatea* markedly ($IC_{50} = 184.32 \pm 1.5 \mu\text{g/ml}$) inhibited the heat induced denaturation of albumin protein in a concentration dependent manner ($r^2 = 0.99$). This effect was comparable to the reference drug ($IC_{50} = 252.46 \pm 0.18 \mu\text{g/ml}$). It is concluded that ARE of *Clitoria ternatea* possesses anti rheumatic arthritic activity and provide scientific justification for its use in ethanomedicine.

Keywords: *Clitoria ternatea*, Rheumatism, Protein denaturation, arthritis, ethanomedicine.

INTRODUCTION

Clitoria ternatea (Family: Fabaceae), butterfly pea in English, Katarolu in Sinhala and Kakkattan in Tamil is an evergreen perennial twining herb with long cylindrical stems which grows up to 2- 3m in height. The plant grows naturally in wild and in gardens in several tropical countries such as India, China Phillipine, Malaysia, Madagascar and Sri Lanka. There are two varieties of this plant: Blue flowered and white flowered^{1,2}. Flowers resemble a conch shell, irregular, bisexual and bear five petals. The leaves are compound, alternate, stipulate and imparipinnate and the root system consists of a fairly stout tap root with few branches and many slender lateral roots³. The main root is thick which grows to more than 2 meters, bears one to several purplish , glaucous nodules. The roots fix nitrogen and have an acrid and bitter taste³. In Ayurveda and traditional and folk medicine in several countries, decoction and extracts made from *C.ternatea* roots are recommended to be used in treatments of several ailments / disorders. These include ingestion, constipation, fever, eye and ear ailments, mucus disorders, sore throat, skin disorders, irritation of bladder and urethra, vaginal disorders, liver disorders, enlargement of abdominal viscera, anxiety, depression, impaired learning and memory, inflammatory conditions¹⁻⁵. It is also used in the treatment of rabies⁵ and rheumatoid arthritic conditions³. Some of these claimed therapeutic effects of *C. ternatea* roots have been experimentally tested by using *in vitro* and *in vivo* studies and have been scientifically validated: such as anxiolytic, antidepressant, learning and memory enhancement, antipyretic, diuretic and anti-inflammatory effect³. However, as for now, antirheumatoid arthritic potential of *C. ternatea* has not been scientifically investigated. This is worth examining since rheumatoid arthritis is a relatively common (prevalence is approximately 1 %) autoimmune disease⁶ and allopathic drugs currently available are

expensive and often associated with unpleasant side effects⁷. As such, there is an imperative need for the development of novel pharmacophores from plant source which are safe potent and cheap. Hence, this study was undertaken to investigate the antirheumatoid arthritic potential of *C. ternatea* roots. This was done using an aqueous hot water extract and *in vitro* bioassay technique.

MATERIALS AND METHODS**Collection and Authentication**

Mature plants were uprooted from a home garden at Beliatta (geographical coordinates: 6°1'119" North, 80°45'2700" East) situated in Hambantota district, Southern province of Sri Lanka in October 2014. The plants were identified and authenticated by Dr (Mrs) S. Ranwala, Department of Plant Sciences, University of Colombo Sri Lanka. A voucher specimen is deposited at Department of Medical Laboratory Science at General Sir John Kotelawala Defence University, Sri Lanka: (CR/01/2014).

Preparation of aqueous root extract (ARE) of *Clitoria ternatea*

The roots were cut off from stems of the plants and thoroughly washed in running tap water. The roots were then air dried in shade for 2-3 days and cut in to small pieces. Twenty three grams of these cut pieces were boiled slowly in 92 ml of distilled water for approximately for 3 hours until the volume is reduced to 18 ml. Light brownish solution was filtered using a muslin cloth (Yield 124.9 %). This ARE was diluted appropriately to obtain the required concentrations: 31.25, 62.50, 125, 250 and 500 µg/ml.

Evaluation of *in vitro* antirheumatoid arthritic action

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 of ARE so that final concentrations become 31.25, 62.50, 125, 250 and 500 µg/ml. Similar volume of double distilled water served as control. Then, the mixtures were (n = 4) incubated at 37 ± 2°C in an incubator (Sanyo, Sanyo Electronic Corporation Ltd, Tokyo, Japan) for 15 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm using a spectrophotometer (Thermo, Thermo Fisher Scientific Ltd., Madison, USA.) by using vehicle as blank. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.50, 625, 1250 and 2500 µg/ml) was used as reference drug and treated similarly for determination of absorbance (n = 4). The percentage inhibition of protein denaturation was calculated by using the following formula^{9,10}.

$$\% \text{ of Inhibition} = 100 \times [Vt / Vc - 1]$$

Vt = absorbance of test sample, Vc = absorbance of control

Phytochemical Screening

The ARE was subjected to qualitative tests for flavonoids, polyphenols, tannings, alkaloids, steroids, terpenoids and amino acids as described by Harry *et al* (1996)⁸.

Statistical Analysis

Table 1: Effect of *Clitoria ternatea* aqueous root extract on *in vitro* heat induced denaturation of protein (n = 4)

Concentration (µg/ml)	Mean Absorbance at 660 nm ± SEM	% of Inhibition
0.00	0.024 ± 0.001	000.00
31.25	0.074 ± 0.009	208.00
62.50	0.122 ± 0.004	408.00
125.00	0.249 ± 0.007	937.00
250.00	0.343 ± 0.012	1329.00
500.00	0.369 ± 0.008	1437.00

Table 2: Effect of Diclofenac sodium on *in vitro* heat induced denaturation of protein (n = 4)

Concentration (µg/ml)	Mean Absorbance at 660 nm ± SEM	% of Inhibition
00.000	0.099 ± 0.002	000.00
78.125	0.122 ± 0.004	23.23
156.25	0.126 ± 0.003	27.27
312.50	0.159 ± 0.005	60.60
625.00	0.302 ± 0.011	205.05
1250.00	0.596 ± 0.013	502.52
2500.00	0.927 ± 0.008	836.36

DISCUSSION

This study examined the *in vitro* antirheumatoid arthritic activity (in terms of inhibition of heat induced protein) of aqueous root extract (ARE) of *C. ternatea*. This was done with a view to scientifically justify its use in traditional and folk medicine as a remedy for rheumatoid arthritis and to investigate the possibility of using the roots of this plant to develop a cheap, safe and potent antirheumatoid arthritic drug. *In vitro* bioassay used to evaluate the antirheumatoid arthritic activity is a widely used, simple, inexpensive, sensitive and a validated model⁹⁻¹¹. Another reason for selecting this *in vitro* bioassay was to avoid the use of live animal models. The results clearly show, for the first time, that ARE of *C. ternatea* possesses marked inhibitory activity against heat induced denaturation of proteins. This indicates that ARE has antirheumatoid arthritic activity^{10,13}. Denaturation of tissues proteins is one of the well known causes of inflammatory and arthritic diseases⁹. Production of autoantigens in certain arthritic conditions are due to *in vivo* denaturation of proteins^{9,12} and several non steroidal drugs

Data is represented as Mean ± Standard Error of Mean (SEM). The extract/drug concentration for 50 % inhibition (IC₅₀) value was calculated using Microsoft Excel 2007 package. Dose dependencies were determined using regression analysis with Minitab 14.0 statistical software. Significance level was set at p < 0.05.

RESULTS

Phytochemical Screening

ARE of *Clitoria ternatea* contained flavanoids, polyphenols, tannings, alkaloids steroids, saponins, terpenoids and amino acids

In vitro antirheumatoid arthritic activity

The results obtained for antiarthritic rheumatoid assay are summarised in Tables 1 and 2. As shown, ARE induced a marked inhibition of protein denaturation activity (ranging from 208 to 1437 % inhibition, (Table 1) with an IC₅₀ value of IC₅₀ = 184.32 ± 1.5 µg/ml. Further, this effect was dose dependent (r² = 0.99, p < 0.05). The reference drug agent, Diclofenac sodium also displayed a profound inhibition of protein denaturation activity (ranging from 23 to 836 % inhibition, (Table 2) with IC₅₀ value of IC₅₀ = 252.46 ± 0.18 µg/ml. This effect too was dose dependent (r² = 0.97, p < 0.05).

used in inflammatory and arthritic conditions impair heat induced denaturation of proteins¹³. Inhibition of heat induced denaturation activity of ARE was dose dependent. This experimental observation indicates that the effect is genuine causal and specific. Further, antidenaturation of protein action of ARE was comparable to reference drug Diclofenac sodium (in terms of IC₅₀ values) which is a well known drug used in the treatment of inflammatory conditions and arthritis⁷. Of interest, inflammation plays a vital role in arthritis⁷. The ARE contained flavonoids, polyphenols, tannings, alkaloids, steroids, saponins, terpenoids and amino acids as major phytoconstituents. As yet, the precise underlying mechanism/s mediating the protein antidenaturation effect by ARE is unknown but could be due to interactions of its poly phenols and flavanoids with aliphatic region around the lysine residue on the albumin protein¹³. 1D and 2D ¹H NMR (One Dimensional and Two Dimensional Protium Nuclear magnetic resonance) studies have shown that agents which have albumin antidenaturation action binds/interacts at these two sites¹³. In conclusion, the

results of this study shows for the first time, that ARE of *C. ternatea* has marked antirheumatoid arthritic properties *in vitro*. It also rationalizes the use of roots of this plant in ethanomedicine as a treatment modality for rheumatoid arthritis. Further, in depth studies are however warranted.

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