Research Article

Effect of *Couroupita guianensis* on Xanthine Oxidase activity and its potent role in treatment of Gout

M. Syed Ali[1*], V. Anuradha[2], R. Keerthiga[1], N. Yogananth[1], H. Sheeba[1]

[1]*PG and Research Department of Biotechnology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai-600119, Tamilnadu, India.*

[2]*PG and Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai-600119, Tamilnadu, India.*

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Abstract

Objective: The aim of the present study was to evaluate the anti-inflammatory activity of fruit extract of *Couroupita guinanensis*. **Materials and methods:** *In vitro* anti-inflammatory activity was evaluated by using human red blood cell membrane stabilization studies. The inhibitory effect of *Couroupita guianensis* on Xanthine oxidase enzyme that promote the oxidation of hypoxanthine to xanthine which converts into uric acid, hence it is much effective in the treatment of gout arthritis. The ethanolic extract of *Couroupita guinanensis* was investigated for *in vitro* anti-inflammatory, albumin denaturation inhibitory activity, xanthine oxidase inhibitory activity. Total phenolics was quantified and bioactive compounds was identified by GC-MS analysis. **Results:** The ethanolic extract shows significant anti-inflammatory activity at the concentration of 400 µg/ml which is comparable to the standard drug 97.63 and fruit extract 79.97% The anti-inflammatory activity of the extracts were concentration dependent, with the increasing concentration the activity is also increased. Concentration of samples was observed at a concentration of 400ug/mL. The *in vitro* albumin denaturation inhibitory activity was compared with carried using Diclofenac, a standard anti-inflammatary drug which showed the maximum inhibition of 82.79% at the concentration of 400 µg/ml compared with control.. **Conclusion:** An enzyme xanthine oxidase inhibitory effect of *Couroupita guianensis* that promoteto the oxidation of hypoxanthine to xanthine which converts into uric acid, hence it is much effective in the treatment of gout arthritis. The ethanolic extract of the *Couroupita guianensis* fruit showed maximum xanthine oxidase inhibitory activity as compared to standard drug allopurinol.

Keywords: Anti-inflammatory, gout, *Couroupita guianensis,* Xanthine oxidase

Introduction

Gout is an acute symptom that is triggered by inflammatory response to monosodium urate crystals mediated by neutrophils and macrophages. It is known to cause inflammatory arthritis in worldwide. Gout is a complex disorder, developed in some people with high count of uric acid in the blood stream, which forms needle like crystals deposited in the joint and develops severe pain, tenderness, swelling and redness. Inflammation is an important component of immune response includes damaged cells, pathogens, mechanical factors, oxygen radicals, angiotensin II, heat shock proteins, coagulation factors, adipokines and platelet products etc. About 6 million men and 2 million women were affected in worldwide. There are some medications like "Diuretic medications" often used for high blood pressure, it has tendency to raise uric acid levels that suppress immune system taken by psoriasis and rheumatoid arthritis patients. This gout is mostly common in men than women. There are various risks factors which includes genes, high cholesterol, high blood pressure, heart disease, diabetes, medications, gender and age, diet such as red meat and shellfish, alcohol consumption, sweet sodas, obesity and person undergone bypass surgery are prone to develop gout (Alexander et al., 2009).

There are some medicinal plants used for the treatment of gout and other hyperuricemia related disorders. *Couroupita guianensis* is a traditional medicine belongs to the family Lecythidaceae, commonly known as

**Address for Corresponding Author:*
M. Syed Ali
PG and Research Department of Biotechnology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai-600119, Tamilnadu, India
E.mail:syedmicro555@gmail.com

"Cannonball tree" or "Sal tree", native to South India and Malaysia. *Couroupita guianensis* produces some bioactive compounds such as couroupitone, glycosides, indirubicin, ketosteroids and isatin. Many researchers have proved that this plant has analgesic properties, anti-inflammatory, antiseptic, antimalarial, antifungal, antimicrobial, antihelmintic, antibiotic and antitumor activity (Gousia et al., 2013). The purpose of this research is to evaluate the efficacy of ethanolic extract of *Couroupita guianensis* to prevent inflammation and its effect on xanthine oxidase activity for treatment of gouty arthritis.

Materials and methods

Collection and extraction of *Couroupita guianensis* fruits

The fruit of *Couroupita guianensis* was collected from in and around Chennai, Tamil Nadu, and India. The collected fruits were dried under shade condition and dried powdered fruit material weighing 25 g was subjected to ethanolic extraction for 48 hrs. The solvent was distilled at lower temperature under reduced pressure and concentrated on water bath to get the crude extract which is stored in dessicator for future use.

In vitro anti-inflammatory activity

The Human Red Blood Cells (Hrbc) membrane stabilization method

The human volunteers were selected and blood sample was taken in absence of NSAIDS intake for 2 weeks prior to the experiment and equal volume of Alsevier solution (2% dextrose, 0.8% sodium citrate, 0.05 % citric acid and 0.42% sodium chloride in water) was mixed and centrifuged at 3,000 rpm. Then the cells were washed with Iso-saline and 10% suspension was prepared. The extracts with various concentrations were prepared (100,200,300, and 400 µ/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml Hyposaline and 0.5ml of HRBC suspension were added, later incubated for 30 min at 37 ºC and centrifuged for 20 min at 3,000 rpm, and haemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560 nm. The standard drug Diclofenac was used as positive control (Sumathi et al., 2016).

In vitro albumin denaturation inhibitory activity of ethanol extract

This assay was carried out by Kumari et al. (2015), in which the each component in the reaction mixtures was reduced by half. The fruit extracts were prepared at concentration of 0.1% each (1.0 mg/ml) and positive standards (Ibuprofen and Diclofenac) as same. A reaction vessel for each mixture was prepared consisted of 200 µl of egg albumin, 1400 µl of phosphate buffered saline, and 1000 µl of the test extract. Distilled water was used as a negative control. The mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. After cooling, the absorbance was measured at 660 nm (Jasco V-630 Spectrophotometer, Japan) and the data were processed by Spectra Manager system. The inhibition percentage of protein denaturation was calculated using the following formula:

$$\% \text{ Denaturation inhibition} = (1-D/C)\times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

Xanthine Oxidase inhibitory activity of fruits

The fruit extracts were prepared at a concentration of 100 µg, 200 µg, 300 µg and 400 µg. The assay mixtures were prepared by adding 300 µl of 50 mM potassium phosphate buffer (pH 7.5), 100 µl sample solutions, 100 µl of freshly prepared XO enzyme solution (0.2 U/ml in phosphate buffer), and 100 µl of deionized water. The positive standard used in the present study was Allopurinol. The mixtures were incubated at 37°C for 15 min. Then 200 µl substrate solution (0.15 mM of xanthine) will be added into the assay mixture and further incubated at 37°C for 30 min. The reaction was stopped by the addition of 200µl of 0.5 M hydrochloric acid. The XO inhibitory activities were assayed spectrophotometrically at 295 nm (indication of uric acid formation at 25°C) using UV/vis spectrophotometer (Jasco V-630 Spectrophotometer, Japan), and the data were processed by Spectra Manager system.. The assay mixture without sample extract served as a negative control. All assays were done in triplicate; thus, inhibition percentages are the mean of three observations. The XO inhibitory activities were expressed as the percentage of inhibition of XO, calculated as follows:

$$\% \text{ XO inhibition} = (1-B/A)\times 100\%.$$

Where B is the absorbance reading of the test sample, and A is the absorbance reading without test sample (negative control). The dose-response graph was utilized to generate a linear equation to estimate the concentration at which maximal inhibition (100%) is obtained (Osman et al., 2016).

Denaturation of total phenolics in ethanol extract

The total phenolic content was estimated by using the Folin Ciocalteu assay. 1ml of standard solution of Gallic acid (100, 200, 300, 400 and 500µg/ml) was added to 25 ml of volumetric flask with 9 ml of distilled water. The reagent blank was prepared and 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. Then 10 ml of 7% NA_2CO_3 solution was added to the mixture after 5 mins. The volume was then made up to the mark. After incubation, optical density was determined at 550 nm with an UV visible spectrophotometer. Total phenolic content

was expressed as mg Gallic acid equivalent (John et al., 2014).

Identification of Bioactive Compounds *(Couroupita guinanensis)* with GC – MS

GC–MS analysis was performed using a Varian CP – gas chromatography. The injected port was heated at 220°C. The injection was performed in split less mode. The carrier gas was helium C- 60 at a constant flow of 1 ml/minute. The over temperature was set at 40°C, then increasing 2°C per minute to 220°C and held for 30 minutes. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under same condition (Sivakumar et al., 2012).

Results

In vitro anti-inflammatory activity was evaluated by using human red blood cell stabilization. The ethanolic extract shows significant anti-inflammatory activity at the concentration of 400 µg/ml which is comparable to the standard drug 97.63% (Table 1 and Figure 1). The anti-inflammatory activity of the extracts were concentration dependent, with the increasing concentration the activity is also increased. Ethanolic extract also exhibited high anti-inflammatory properties determined by albumin denaturation studies. In all the assays, the final concentration of samples was 400ug/mL. The *in vitro* albumin denaturation inhibitory activity was compared with carried using Diclofenac, a standard anti-inflammatary drug which showed the maximum inhibition of 82.79% at the concentration of 400 µg/ml compared with control (Table 2 and Figure 2).

Table 1. *In vitro* anti-inflammatory activity of ethanolic extract

Concentration (µg/ml)	% inhibition	
	Diclofenac	Test sample
100	68.60	65.38
200	78.25	69.89
300	88.04	70.14
400	97.63	80.97

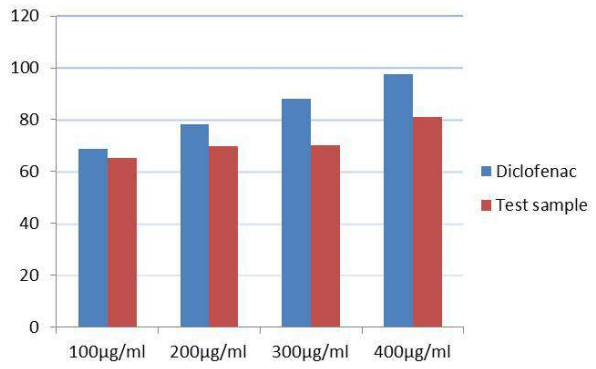

Figure 1. *In vitro* anti-inflammatory activity of ethanolic extract

Table 2. *In-vitro* Albumin denaturation inhibitory activity of ethanolic extract

Concentration (µg/ml)	% inhibition	
	Diclofenac	Test sample
100	75.50	69.38
200	67.30	72.89
300	59.74	76.14
400	67.21	82.97

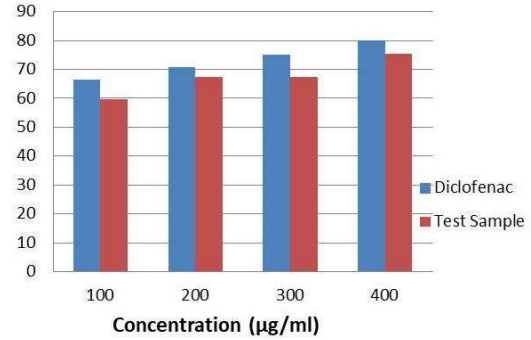

Figure 2. *In-vitro* Albumin denaturation inhibitory activity of ethanol extract

However, when tested using the xanthine oxidase assay, the extract also exhibited moderate antigout properties with maximum inhibition 79.97% inhibition and low inhibition took place for the leaves extracts (Table 3 and figure 4).

Table 3. *In-vitro* XO inhibitory activity of ethanol extract

Concentration (µg/ml)	% inhibition	
	Xanthine	Test sample
100	75.50	59.74
200	67.30	67.30
300	59.74	73.4
400	67.21	79.97

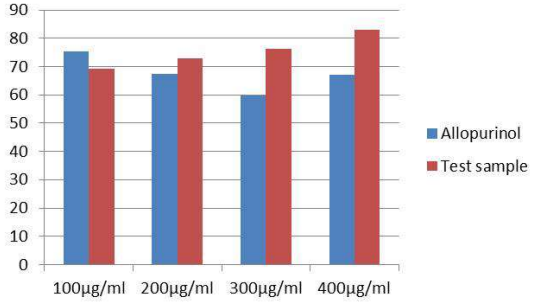

Figure 3. *In vitro* XO inhibitory activity of ethanol extract

The total phenolic content expressed in terms of GAE was found to be 400ml of GA/g and 8.69% (w/w) respectively. The total phenolic contents were calculated

using the following linear equation based on the calibration curve of gallic acid seen in (Figure 4).

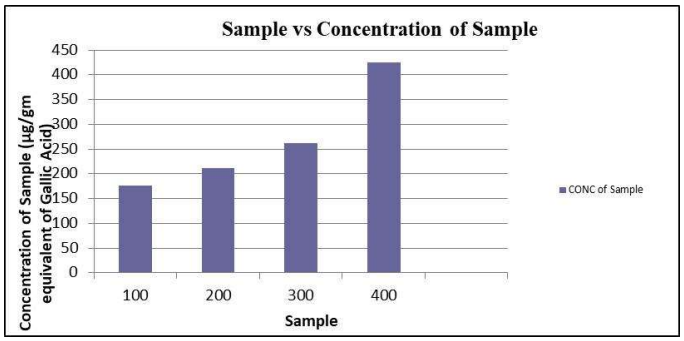

Figure 4. *In vitro* Total phenolic activity of ethanol extract

The partially purified bio active compounds were characterized with the help of GC-MS analysis. Based on this analysis the responsible compounds for antimicrobial activity were found to be Benzene Benzoate. In this analysis various peaks were observed at the retention time of 8-23 minutes. The peaks were compared with the existing data available from the library was searched and the maximum similarity was showed with benzyl benzoate, the simplest aromatic compounds (Figure 5).

Discussions

The fruit extract exhibited membrane stabilization effect by inhibiting hypo tonicity induced lysis of erythrocyte membrane. Antiinflammatory compounds can act on many steps of pathophysiological process. In addition, a compound can either act by inhibiting the release of preformed stored mediators or by blocking mediator receptor interaction on target cells. An anti-inflammatory compound may also act by immune stimulation that in turn promotes an increase removal of the insulting signal molecules, which results in a less aggressive inflammatory response to allergen challenge (Safaihy and Sailer, 1997; Labuet al, 2015). Even though pharmacological industries have produced a number of new antibiotics, in the last three decades, resistant to these drugs by microorganisms have been increased. To control the use of antibiotics, we develop research to better understand the genetic to develop new drugs either natural or synthetic. The ultimate goal is to offer appropriate and efficient antiinflammatory drugs to the patient.

Inflammation is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial or heterogeneous nature of the diseases increases so does the need for more challenging and appropriate therapies. Inflammation is a common phenomenon and it's a reaction of living tissues towards injury. NSAIDs possibly induce the redistribution of lymphocytes which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. The lysosomal enzymes released during

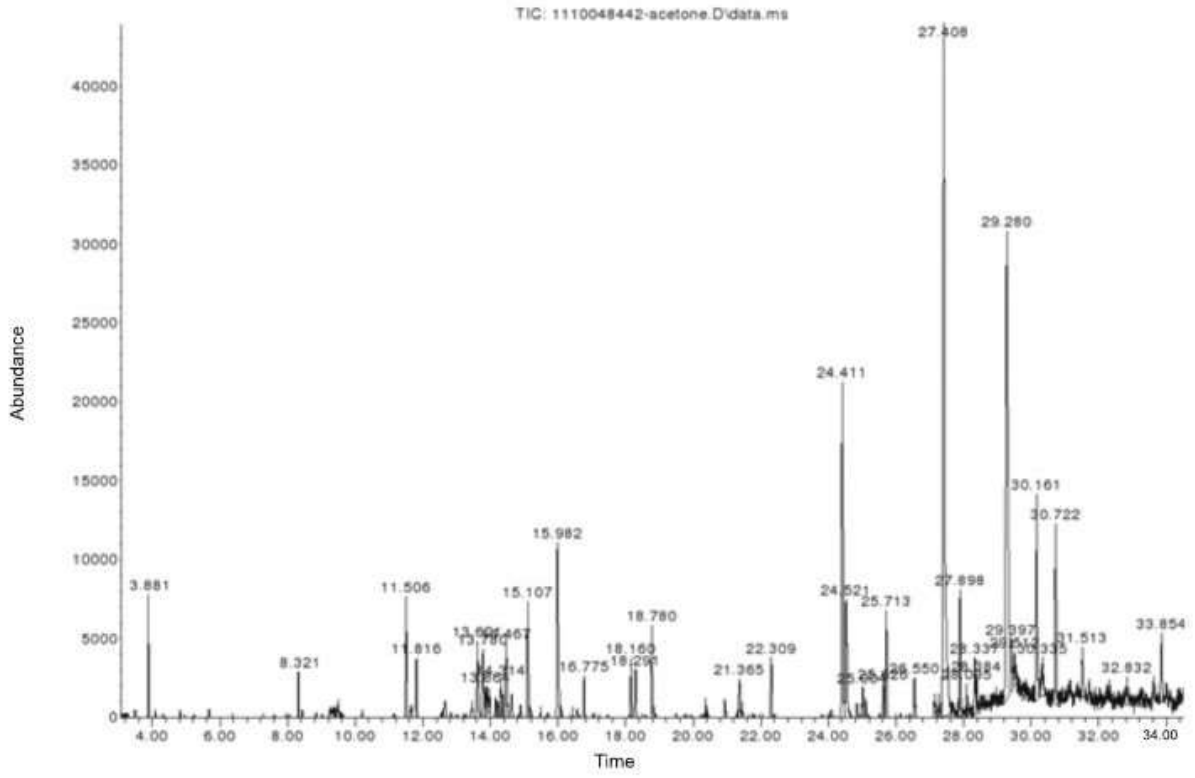

Figure 5. Identification of Bio Active Compounds by GC-MS Analysis

inflammation produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic by inhibiting the Lysosomal membrane (Chavda, 2015). The majority of these trees outside their natural environment have been planted as a botanical curiosity, as they grow very large with distinctive flowers. The genus *Couroupita* represents more than 30 recognized species throughout the world. In French it is known as Calabasse Colin. It is native to South India and Malaysia. The Puducherry Government has announced cannon ball flower (Nagalingam flower) as the State Flower (Satyavati, 1976).

In recent years, there is an increasing interest in finding herbal plants and phytochemicals which possess the capacity to inhibit xanthine oxidase activity and reduce urate levels. Longan is a fruit used in herbal preparations in China, and though unpollinated longan flowers and nonedible fruit seeds are generally regarded as disposable byproducts, studies show that longan flowers, pericarps, and seeds contain high levels of phenolics and flavonoids, which exhibit high antioxidant activity and may be rendered suitable as protective agents (Rastogi et al., 1995). It can be used for kidney disorder associated inflammation of join and there by prevention of gout arthritis (Khan et al., 2003; Umachigi et al., 2007; Mariana et al., 2010).

Even though pharmacological industries have produced a number of new antibiotics, in the last three decades, resistant to these drugs by microorganisms have been increased. To control the use of antibiotics, we develop research to better understand the genetic to develop new drugs either natural or synthetic. The ultimate goal is to offer appropriate and efficient anti inflammatory drugs to the patient.

Conclusion

This is the first comparative *in vitro* study on anti-inflammatory activity of *Couroupita guianensis* fruit. The current study provides evidence for the traditional use of *Couroupita guianensis* against inflammatory disorders. The ethanolic extract of the *Couroupita guianensis* fruit showed maximum anti-inflammatory activity and Xanthine oxidase enzyme inhibition as compared to standard drug and CGEF extract. Thus further investigation would be carried out in isolation of the active compounds and elucidate their inhibitory mechanism in *in-vivo*.

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