

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



E-ISSN: 2278-4136 P-ISSN: 2349-8234 JPP 2018; 7(2): 877-879 Received: 10-01-2018 Accepted: 12-02-2018

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Phytochemical analysis of fruit pulp of Couroupita guianensis Aubl.

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Abstract

Couroupita guianensis Aubl. A Lecythidaceae member is analyzed to estimate the phytochemicals and to evaluate the anti-inflammatory, anti-arthritic and anti-oxidant activities. Studies reveal that *C. guianensis*. Aubl. Is a source of medicinally active compounds and have various pharmacological effects.

Keywords: Couroupita guianensis Aubl, cannon ball tree anti-inflammatory, anti-arthritic, anti-scavenging

1. Introduction

Plants are recognized in the pharmaceutical industry due to their broad spectrum of structural diversity and their wide range of pharmacological activities. The biologically active compounds that are present in plants referred to as *phyto-chemicals* (Krishnanadha P Ingle *et al.*, 2016) ^[5]. Phytochemistry or plant chemistry concerned with the enormous variety of organic substances that are accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function (J.B. Harborne, 1980) ^[4]. Scientists estimate that there may be as many as thousand different phytochemicals having potential to affect diseases such as cancer, stroke or metabolic syndrome.

Since the phytochemicals cure diseases without causing any harm to human beings; these can also be considered as "man friendly medicines". This paper mainly deals with collection, extraction, qualitative and quantitative analysis of phytochemicals present in the fruit pulp of *Couroupita guianensis* Aubl.

Couroupita guianensis Aubl.

Couroupita guianensis Aubl. Commonly known as cannon ball tree grown in Indian gardens as an ornamental tree for its beautiful flowers. It also known as Nagalingam tree in Tamil and kailaspati in Hindi (Vivek P Chavda, 2015) [9]. The trees are grown extensively in Shiva temples in South India. Hindus revere it as a sacred tree because the staminal sheath resembles the hood of Naga, a sacred snake, protecting a shivalingam; represented by reduced stigma. Hence the name 'Nagalingam tree'. The cannon ball tree so called because of its brown cannon ball like fruits. The majority of these trees outside their natural environment have been planted as a botanical curiosity, as they grow very large with distinct flowers. The genus couroupita represent more than 30 recognized species throughout the world (Vivek P Chavda, 2015) [9]. In French it is known as Calabasse Colin. It is native to south India and Malaysia. The Puducherry government has announced cannon ball flower as the state flower (Deepa, 2007)

The fruit contains small seeds in a white unpleasant smelling jelly, which are exposed when the upper half of the fruit goes off like a cover. The hard shells are used to make containers and utensils (Vivek P Chvda, 2015) ^[9]. Cannon ball flowers are considered of special significance in Buddhist culture in Srilanka (Shah G.N *et al.*, 2013) ^[7]. The long dangling fruity branches give the tree an unkempt appearance (Dr. Mahipal Singh, 2014) ^[6].

Traditional Uses of Couroupita guianensis Aubl.

The trees are used to cure cold and stomach ache. Juice made from leaves is used to cure skin diseases and Shamanas of South America have even use tree parts for treating malaria, while the flowers are used to cure cold, intestinal gas formation and stomach ache (Velliangiri Prabhu *et al.*, 2012) [8]. The fruit pulp can disinfect wounds and young leaves ease tooth ache. Bark used to treat hypertension, tumors, pains and inflammatory process (Dr. Mahipal Singh, 2014) [6]. The fresh fruit pulp is used in the preparation of cooling medicinal drink and cure head ache. Leaves are widely used as an analgesic medicine by the rural population worldwide

Correspondence Y Reshma Department of Botany, S.D. College, Alappuzha. Kerala, India (Geetha *et.al*, 2005) ^[3]. This plant is very important in traditional veterinary medicine. Cold relief bam, flower dried and powered used as snuff, cure scabies, bleeding piles, scorpion poison, dysentery, arthritis, fragrance of flowers used for curing asthma (Velliangiri Prabhu *et al.*, 2012) ^[8].

2. Materials and Methods

Collection of fruit sample and identification of the specimen

The fruits of *Couroupita guianensis* Aubl is collected from the premises of S.D. College, Alappuzha. The plant was identified with the help of Floras and, in consultation with the experts in angiosperm taxonomy.

Collected fruit is subjected to different processes for studying its activity.

Anti-inflammatory activity

The anti-inflammatory activity of plant extracts was assessed by in vitro HRBC membrane stabilization method. Fresh whole human blood (10ml) was collected and transferred to the heparin zed centrifuged tubes. The collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with isosaline (0.85 %, dissolve 8.5g NaCl in water. Autoclave 15 min at 121°C. Cool to room temperature). To 1mL of HRBC suspension, equal volume of plant extracts in three different concentrations (25, 50, 100 µL) was added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage of haemolysis was calculated then by the formula as given below:

Percent of hemolysis = OD of test/OD of control $\times 100$

The percentage of protection can be hence calculated from the equation as given below:

Percent of protection = 100-OD of test/OD of control $\times 100$

Anti-arthritic activity

0.5% Bovine Serum Albumin (BSA): Dissolved 500mg of BSA in 100 ml of water.

Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5% W/V aqueous solution) and test solution of various concentrations. (Stock-10mg/ml) Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (0.5% W/V aqueous solution) and 0.05ml of distilled water. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.

Procedure: 0.05 ml various concentrations of test samples were taken mixed with 0.45 ml (0.5% w/V BSA). The samples were incubated at 37°c for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm. The control represents 100% protein denaturation. The percentage inhibition can be calculated as.

Percentage Inhibition= (optical density of control – optical Density of test)/optical density of control}/ \times 100]

Abts scavenging effects (Shirwaikar et al. 2006) [1].

ABTS radical cations (ABTS+) were produced by reacting

ABTS solution (7mM) with 2.45mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the three different extracts were added to 0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm in a spectrophotometer (Genesys 10-S, USA) and the per cent inhibition was calculated using the formula,

% of Inhibition =
$$\frac{\text{Control - Test}}{\text{Control}}$$

3. Result and Discussion

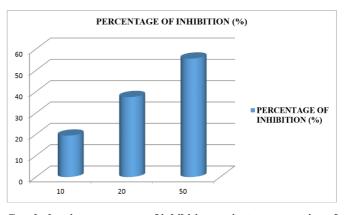
The result of phytochemical analysis of *Couroupita guianensis* Aubl. Which involved the qualitative determination of flavanoids, tannins, terpenoids, steroids, cardiac glycosides, alkaloids, phenols and carbohydrate. Amino acid and sterols found to absent in the sample through preliminary investigation. The free radical scavenging activity of solvent was assured by ABTS assay. Anti-inflammatory activity of the sample was assessed by in vitro HRBC membrane method. Anti-arthritic activity was confirmed by BSA (Bovine Serum Albumin) test.

Anti-inflammatory activity

The analysis of the fruit pulp shows that it has anti-inflammatory activity. It shows increase in the concentration is increasing the anti-inflammatory activity, which inhibit the inflammatory effect.

Sample Name	Concentration (mg)	Percentage of Inhibition
Fruit	10 mg	19.44
	20 mg	37.50
	50 mg	55.56

Anti-inflammatory activity



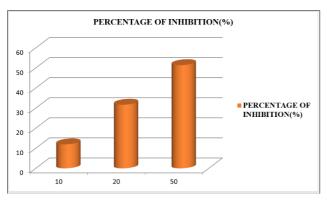
Graph showing percentage of inhibition against concentration of sample in mg

Anti-arthritic activity

The percentage of anti-arthritic activity shows that which is very effective in the controlling arthritis. The percentage of inhibition is gradually increased with the increase in concentration.

Sample Name	Concentration (mg)	Percentage of Inhibition
Fruit	10 mg	11.96
	20 mg	31.70
	50 mg	51.43

Anti-arthritic activity



Graph showing percentage of inhibition against concentration of sample in mg

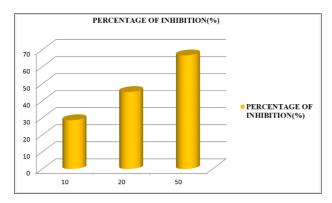
Abts scavenging effects

The analysis also showed that the pulp has ABTS scavenging effect on increase in concentration of the pulp.

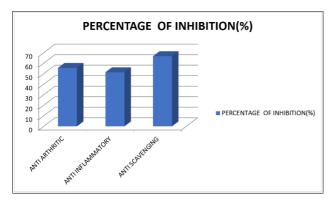
Sample Name	Concentration (mg)	Percentage of Inhibition
	10 mg	28.66
Fruit	20 mg	45.22
	50 mg	66.72

Anti-scavenging activity

Fruit pulp sample of *Couroupita guianensis* Aubl. exhibited good anti-oxidant activity, 50mg of extract showed 66.72% of inhibition on ABTS scavenging assay.10 mg of sample exhibits 28.66% of inhibition and 20 mg of sample shows 45.22% of inhibition. Percentage of inhibition increases with increase in concentration of sample. ABTS radical scavenging graph shows a positive correlation between concentration of sample and anti-scavenging activity.



Graph showing percentage of inhibition against concentration of sample in mg



Graph showing anti-inflammatory, anti-arthritic, antiscavenging activity in 50mg sample

Bar diagrams plotted the percentage of inhibition against various concentration of the sample (10mg, 20mg, and 50mg). The graph compares anti-inflammatory, anti-arthritic and antiscavenging activity of sample in the same concentration of sample. Graph shows that the percentage of inhibition is directly proportional to the concentration of sample in all the three cases. Results reveals that the material possess anti-inflammatory, anti-arthritic and anti-scavenging activities. Fruit pulp extract of *Couroupita guianensis* Aubl. shows high amount of anti-scavenging activity than anti-arthritic and anti-inflammatory activity.

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