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Bioactive Potential of *Couroupita guianensis* Aubl. : Antioxidant and Anti-inflammatory Activities

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Abstract

Several plants have been used traditionally in Ayurvedic medicine for the management of a wide range of medical conditions. Furthermore, as natural resources thought to be rich in nutritional and pharmacological qualities to treat and cure a variety of diseases, multipurpose medicinal plants continue to attract research interest and commercial attention worldwide. The present study was designed to evaluate the antioxidant potential and antibacterial activity of ethanol extract of leaves and flowers of *Couroupita guianensis*. An ethanol extract of *Couroupita guianensis* leaves and flowers were obtained by the hot percolation method. The extracts were tested for a preliminary phytochemical screening by standard methods. *In vitro* antioxidant activity was assessed by 1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay, by using Ascorbic acid as the standard antioxidant. *In vitro* anti-inflammatory activity of the extracts were measured by protein denaturation assay, by using aspirin as standard. Phytochemical analysis of the extract showed the presence of major classes of phytochemicals alkaloids, phenolic compounds such as flavonoids, tannins and saponins. *Couroupita guianensis* ethanolic extract showed the highest reducing capacity. The antioxidant and anti-inflammatory activity of the extracts was found significant. The results of our study suggest that leaves of *Couroupita guianensis* possess potent antioxidant and anti-inflammatory and are a good source of natural antioxidants.

Keywords: *Couroupita guianensis*, leaf, flower, antioxidant, anti-inflammatory

1. INTRODUCTION

Herbal medicines play an important role in the treatment throughout the world. The plant has great therapeutic association in Indian system of medicine. The natural medicines can be readily contaminated with or replaced entirely with low quality constituents in order to satisfy the growing demand. Therefore, it is critical to set guidelines for the authentication of potent medicinal plants that are developed to treat diseases. Pharmacognostic studies will provide standard guidelines and confirm plant identity, hence contributing to the prevention of adulteration. *Couroupita guianensis* (Aubl) commonly known as cannon ball tree which belongs to the family Lecythidaceae. It is also known as Naglingam in Tamil and Kailashpati in Hindi. It habitually seen in Lord Shiva temples in South India and is considered a holy tree. It is a giant tree with vibrant and intensely scented flowers. The fruits are large and rounded in shape. Due to shape and size of the fruits, the plant is commonly called as 'Cannon ball tree'. The fruits have acidic pulp inside and hard shell around it. The fruits are edible and have properties like neuro-pharmacological, wound-healing, hepato-protective, anti-nociceptive, immune-modulatory, antiulcer, antioxidant, antimicrobial, cyto-protective, anti-inflammatory,

anti-hyperglycemic and antidiabetic. Traditionally the plant is used in the treatment of stomach ache, skin diseases, malaria, cold, headache, asthma, arthritis, scorpion bite and dysentery. Bark is used in the treatment of hypertension, inflammatory diseases and tumours. Leaves are used as an analgesic and leaf juice used in treatment of skin diseases in South America. Fresh fruit pulp used for making cooling medicinal drink, pulp can disinfectant wounds as it has antibacterial properties. The fragrance of flowers is used for curing asthma and the shell of the fruit is used as a utensil. The study of this plant's pharmacological characteristics has grown exponentially in the last few years. Antioxidant components are micro constituents that scavenge free radicals and prevent oxidizing chain reactions from starting or spreading, thereby preventing lipid oxidation. The use of antioxidants in clinical settings has grown significantly in recent years for the treatment and management of chronic degenerative diseases, aging, and neurodegenerative disorders (Harborne et al., 1998). In view of the above, we aimed this study to assess the antioxidant potential and anti-inflammatory effect of *Couroupita guianensis* [1, 2, 3].

2. Materials and Methods

2.1 Sample Collection

The healthy and fresh leaves and flowers were collected from Chennai. The samples were carefully washed under tap water to remove dust and dried under shade. The dried material coarsely powdered and kept in air-tight containers for further study.

2.2 Preparation of extracts

The leaf and flower extract were separately with ethanol as solvent by hot percolation method using soxhlet apparatus. Then the ethanol extract was concentrated, and weighed. The dry extract was stored at 4°C until used.

2.3 Qualitative phytochemical screening

Phytochemical analysis of ethanolic extracts of leaf and flower were carried out by qualitative test according to standard methods. The extracts were screened for alkaloids, flavonoids, steroids, carbohydrates, saponins, tannins, proteins and phenols [4, 5].

Test for alkaloids

Mayer's test: To the 1 ml test solution, Mayer's reagent (Mercuric-potassium iodide) was added. Creamy precipitation showed the presence of alkaloids.

Test for flavonoids

Shinoda test: Magnesium chips were added to the extract followed by addition of conc. HCl. Reddish pink showed the presence of flavonoids.

Test for steroids

Libermann-Buchard test: Few drops of acetic anhydride, 2 ml chloroform and few drops of conc. H_2SO_4 was added to the test solution. Formation of green colour indicated the presence of steroids.

Test for carbohydrates

Benedict's test: To the 2 ml test solution, 0.5 ml Benedict's reagent was added. Mixed it well and boiled for 2 minutes. A green colour showed the presence of carbohydrates (reducing sugar).

Test for saponins

Foam test: 5 ml distilled water added to the 3 ml of extract. Foam formation indicated the presence of saponins.

Test for tannins

Lead acetate test: 1% lead acetate was added to 2 ml of extracts. A yellow precipitation indicated the presence of tannins.

Test for proteins

Biuret test: To the 1 ml test solution, 2 ml of biuret reagent (2 drops of 1% CuSO4 + 1 ml of 40% NaOH) was added. The violet colour indicated the presence of proteins.

Test for Phenols

Folin-Ciocalteu's –reagent test: A few drops of Phenol-reagent Ciocalteu's were added to 1 mL of the extract, accompanied by a few drops of Na_2CO_3 solution (15%). The presence of phenols is indicated by the production of a blue or green tint.

2.4 *In vitro* Anti-Oxidant Activity

2.4.1 DPPH Free Radical-scavenging Activity

The Free Radical Quenching Activity of extracts, was systematically evaluated using the Free Radical Quenching assay, following established protocols [6,7]. This assay measures the ability of compounds to provide hydrogen atoms by monitoring the reduction of a methanol solution containing 2, 2-diphenyl-1-picrylhydrazyl (DPPH), which changes colour from violet/purple to yellow in the presence of antioxidants.

Aliquot 3.7ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank. Add 100µl respective sample concentration of extracts to all other tubes marked as tests. Each experiment was performed in triplicate. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm.

Calculation

$$\textbf{Inhibition (\%)} = \frac{\textbf{OD of Control} - \textbf{OD of Experiment}}{\textbf{OD of Control}} \times 100$$

2.4.2 ABTS free radical scavenging activity

The total antioxidant activity determined by radical cation ($ABTS^{\bullet+}$) decolonization assay was performed according to Zhao et al., 2011 and Olajire & Azeez, 2011 with slight modifications. A 7 mM of ABTS was mixed with double distilled water and 2.4 mM potassium persulfate to prepare the ABTS reagent. This mixture was kept in a dark place for 13 to 17 h. The concentrated solution was diluted with methanol and adjusted to get an absorbance of 0.7 at 734 nm before analysis.

To measure the antioxidant power of the extracts, 3 ml of $ABTS^{\bullet+}$ reagent solution and 1 mL of each extract at various concentration levels (ranging from 20-100 µg/ml) were mixed and the absorbance was then measured at 734 nm [8,9].

Calculation

$$\textbf{Inhibition (\%)} = \frac{\textbf{OD of Control} - \textbf{OD of Experiment}}{\textbf{OD of Control}} \times 100$$

2.4.3 Ferric reducing antioxidant power assay

The antioxidant capacity of the extracts were evaluated using the FRAP method following the procedure outlined by Oyaizu, 1986 (10). The extracts were prepared at concentrations ranging from 20 to 100 µg/mL.

1mL of each extract were mixed with 2.5 mL of phosphate buffer (0.2 mM, pH 7.4) and 2.5 mL potassium ferricyanide. The solution was incubated at 50°C for 20 min. After the incubation, 2.5 mL 10% trichloroacetic acid (TCA) was added and centrifuged at 3000 rpm for 10 min. The clear supernatant (2.5 mL) was gently diluted with the same volume of double distilled water and treated with 0.1% W/V ferrous chloride. The absorbance was measured at 700 nm.

2.5 *In vitro* Anti-inflammatory Activity

2.5.1 Inhibition of albumin denaturation

The anti-inflammatory activity of the extracts was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima *et al.*, 1968 and Sakat *et al* 2010 followed with minor modifications. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660nm [11,12].

Calculation

% inhibition = (Abs Control –Abs Sample) / Abs control X 100

2. Results

3.1 Preliminary Phytochemical analysis

A preliminary phytochemical analysis of *Couroupita guianensis* Aubl leaves and flowers were performed in ethanol extract by using various qualitative tests. The results of the tests are shown in Table 1.

Table 1 Preliminary phytochemical analysis of *Couroupita guianensis* Aubl leaves and flowers

S.No.	Secondary metabolites	Test	Leaf	Flower
1	Alkaloids	Mayer's test	+	+
2	Flavonoids	Shinoda test	+	+
3	Steroids	Libermann Burchard test	+	+
4	Carbohydrates	Benedict's test	+	-
5	Saponin	Foam test	+	-
6	Tannins	Lead acetate	+	+
7	Proteins	Biuret test	-	-
8	Phenols	Folin- Ciocalteu's reagent test	+	+

+ indicates presence of secondary metabolite

- indicates absence of secondary metabolite

3.2 In-*vitro* antioxidant activity

3.2.1 DPPH Free Radical-scavenging Activity

The antioxidant activity of leaf extracts has been studied by its ability to reduce DPPH. Interaction of antioxidant compounds present in the leaf and flower extract with DPPH is based on the transfer of hydrogen atom or electron to DPPH radical and converts it to 1, 1- diphenyl-2- picrylhydrazine. The result of reduction DPPH radicals causes discoloration from purple color to yellow pale color which demonstrates the scavenging activity. The antioxidant activity of the extracts and ascorbic acid against DPPH assay was tested with concentrations ranging from 20 to 100 µg/ ml and the results are shown in Table 2.

As shown in the table 2, both the leaf and flower extracts were exhibited a comparable and significant concentration-dependent free radical scavenging activity from 21.67% to 59% and 15.43% to 48.32%,

respectively, compared with that of the standard Ascorbic acid 35.92% to 98.40%. The IC_{50} values of DPPH assay for the leaf and flower extracts were 80 µg/ml and 100 µg/ml, respectively. While the standard had an IC_{50} of 40 µg/ml. Our results indicates that the leaf extract has higher antioxidant than that of flower extract.

Table 2 DPPH Scavenging Assay

S.No.	Concentration (µg/ml)	% inhibition		
		Leaf Extract	Flower Extract	Ascorbic acid
1	20	21.67	15.43	35.92
2	40	28.73	21.23	**52.84**
3	60	39.28	36.76	69.53
4	80	**47.59**	41.63	91.72
5	100	59.22	**48.32**	98.40

3.2.2 ABTS free radical scavenging activity

The ABTS assay provided further insights into the antioxidant capabilities of both the leaf and flower extracts of *Couroupita guianensis* Aubl. The extracts were tested at various concentrations, along with ascorbic acid (standard) at concentrations ranging from 20-100 µg/ml. In comparison, the ethanolic extract of leaf exhibited significantly higher activity than the flower extract, with 48.31% scavenging at 60 µg/mL (IC_{50}). These findings indicate that both the extracts shows better antioxidant potential.

Table 2 ABTS Assay

S.No.	Concentration (µg/ml)	% inhibition		
		Leaf Extract	Flower Extract	Ascorbic acid
1	20	27.22	22.58	**57.22**
2	40	35.83	34.40	67.83
3	60	**48.31**	43.01	78.31
4	80	55.90	**54.83**	85.90
5	100	62.78	66.66	95.78

3.2.3 Ferric reducing antioxidant power assay

The ferric-reducing power of the ethanolic extracts of leaf and flower of *Couroupita guianensis* Aubl were assessed at concentrations between 20 and 100 µg/mL. Both extracts showed strong reducing capacities.

Table 2 FRAP Assay

S.No.	Concentration (µg/ml)	% inhibition		
		Leaf Extract	Flower Extract	Ascorbic acid
1	20	69	57	140
2	40	75	68	150
3	60	82	80	166
4	80	90	94	184
5	100	105	110	196

3.3 *In vitro* Anti-inflammatory Activity

The anti-inflammatory activity of *C.guianensis* Aubl leaves and flowers extracts were evaluated against denaturation of albumin method. There was significantly higher inhibition was seen in leaf extract as compared to flower extract. The maximum percentage of inhibition reported was 64.36% and 51.09% for leaf and flower extract, respectively. The corresponding IC_{50} values were determined as 60 µg/mL and 100 µg/mL for leaf and flower extract, respectively. Based on the findings, both the extracts of *C.guianensis* Aubl demonstrated the significant inhibition of albumin denaturation caused by heat compared to standard Aspirin, which showed an inhibition of 98.48% with an IC_{50} value of 40 µg/mL.

Table 3 In-vitro anti-inflammatory activity

S.No.	Concentration (µg/ml)	% inhibition		
		Leaf Extract	Flower Extract	Aspirin
1	20	35.05	23.54	71.15
2	40	42.22	31.99	77.69
3	60	**49.87**	39.57	89.55
4	80	58.53	46.35	93.33
5	100	64.36	**51.09**	98.48

3. Discussion

The phytochemical screening assays conducted on the leaves and flowers extracts of *C.guianensis* Aubl, yielded a significant insights into their secondary metabolite composition. The qualitative analysis revealed the presence of bioactive compounds such as alkaloids, flavonoids, steroids, carbohydrates, saponins, tannins and phenols in leaf extract. However, flower extract revealed the presence of alkaloids, flavonoids, steroids, tannins and phenols. These findings align with earlier research where similar phyto-chemicals were reported in *C.guianensis* Aubl, and other medicinal plants, indicating the extract's potential therapeutic uses [13].

Many studies on other medicinal plants such as *Moringa oleifera, Camellia sinensis* and *Azadirachta indica* that the presence of phenols and flavonoids, contributed significantly to free radical scavenging, anti-inflammatory, and anti-microbial activities. Medicinal plants contain natural antioxidants, which are in charge of preventing the adverse consequences of oxidative stress. Many plant extracts have effective antioxidant qualities because of their phytoconstituents, which include flavonoids and phenols. Polyphenols and other antioxidants have been screened using this technique on a large scale. It has been reported that phenolic compounds contribute to the majority of the antioxidant efficacy in natural sources [14].

Generally, polar solvent extracts exhibited significantly improved antioxidant capacities, attributed to their higher yields of phenolic compounds [15]. All these results indicate that the ethanolic extracts of leaves and flowers of *C.guianensis* Aubl exhibits a potent anti-inflammatory and antioxidant activities and thus could further be explored for its effectiveness as a therapeutic agent.

CONCLUSION

The present study concludes that the ethanolic extracts of leaves of *Couroupita guianensis* Aubl contain high antioxidant and anti- inflammatory property, than its flower extract. The presence of phytochemicals such as alkaloids, flavonoids, steroids, carbohydrates, saponins, tannins and phenols in leaf extract was revealed in the preliminary phytochemical assay, while the flowers showed presence of alkaloids, flavonoids, steroids, tannins and phenols. The ethanolic extracts of leaves and flowers of *Couroupita guianensis* Aubl demonstrated the promising anti-inflammatory and antioxidant properties, making them potential candidates to be used in therapeutical applications. However, further detailed studies are needed in this direction to explore the utility of *Couroupita guianensis* Aubl total extracts or isolation of its active ingredients to gain further insight into the mechanism of its action as a natural remedy with therapeutic applications.

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