

EXTRACTION OF FLAVONOIDS FROM VARIOUS PARTS OF *COUROUPITA GUIANENSIS* AND ITS EFFICACY AGAINST PATHOGENIC BACTERIA

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Received: 30 December 2016, Revised and Accepted: 14 January 2017

ABSTRACT

Objective: Quantitative, qualitative, high performance liquid chromatography (HPLC) analysis of flavonoids content from different parts of *Couroupita guianensis* and antibacterial activity against various pathogenic bacteria.

Methods: Different parts of *C. guianensis* such as leaves, stem bark, flower anther, flower petals, fruit rind, and fruit pulp were collected and used for extraction of active constituents. The phytochemical analysis was conducted to identify the presence of flavonoids in different plant parts. The amount of flavonoids were analyzed by quantitative analysis. Furthermore, HPLC analysis was performed to isolate and identify the flavonoids from the different parts. The methanol extract of different plant parts was also used to test the antibacterial efficacy in different human bacterial pathogens.

Results: Flavonoids demonstrate a wide range of biochemical and pharmacological effects. In this study, we identified the flavonoids content in different parts of *C. guianensis*. The HPLC analysis has significantly proved the presence of flavonoids in different plants parts and amount of flavonoids differs in different parts of the plant. The results also showed the significant efficacy of its extract to the different pathogenic bacterial strains.

Conclusion: Our study suggested that *C. guianensis* is a richer source of flavonoids and due to a higher amount of flavonoids; it may have enormous potential to scavenge the free radicals, oxidative damage of cell during different stresses including bacterial infections. We also suggested that HPLC analysis is efficient method to isolate and identify the different compounds and study can further extend to identify and isolate the different novel compounds from *C. guianensis*.

Keywords: *Couroupita guianensis*, High performance liquid chromatography, Flavonoids, Antibacterial activity.

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INTRODUCTION

Plants are the basic source of knowledge of modern medicine. Almost all the parts of the plant, namely leaves, flowers, fruits, roots, stem, and seeds are known to have various medicinal properties. The trend of using natural products has increased, and the active plant extracts are frequently screened for new drug discoveries. The use of the medicinal herbs for curing disease has been documented in the history. Herbal drugs are prescribed widely because of their effectiveness, less side effects, and relatively low cost [1]. Therefore, investigation on some active principles from traditional medicinal plants has become more important [2]. The world health organization [3] has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs [4].

The *Couroupita guianensis* (Aubl) family Lecythidaceae commonly known as cannon ball tree, locally known as "kailashpati" is found throughout India. It is widely cultivated for its large showy flowers and reddish brown woody capsular fruits up to 20 cm in diameter. It is grown in Indian gardens as an ornamental tree. It is native to South India and Malaysia and commonly known as Nagalingapushpam in Tamil. Traditionally, the leaves of this plant have been used in the treatment of skin disease. Native amazonian people used the infusion or tea obtained from leaves, flower and bark of *C. guianensis* to treat hypertension, tumors, pain, and inflammatory processes [5].

In Orissa decoction of flowers has been used to boost the immune system to fight number of disease [6,7]. The flower extracts of this plant had been screened for larvicidal activity against vector [8] and immune modulatory activity [9]. *C. guianensis* has showed a broad spectrum of antibacterial and antifungal activities [10]. It is known that one of the

active constituents of the medicinal plant *C. guianensis* namely isatin, is known to exert cytotoxic activity against certain cancer cell lines, being a potential source of new chemo therapeutic agents [11].

The *C. guianensis* leaves are rich in phytochemicals and reported to contain quercetin, saponin, and tryptanthrin [12]. The tree is also rich in providing anthocyanin, flavonoid, volatile constituents like eugenol and farsenol. From the flowers of *C. guianensis* stigma sterol and aliphatic hydrocarbon have been isolated [13].

Therefore, the main objectives of this study to qualitative and quantitative analysis of flavonoids from methanolic extract of the leaves, stem bark, fruit and flower of *C. guianensis*, and high performance liquid chromatography (HPLC) analysis of flavonoid content of the different plant parts and antibacterial efficacy against human pathogen.

METHODS**Plant collection and identification**

The samples of *C. guianensis* were collected from the B S Abdur Rahman University campus near to the life sciences block. The *C. guianensis* plant was identified and authenticated by the standard taxonomic characteristic features (keys) according to the flora of madras presidency [14] and the flora of Tamil Nadu Carnatic [15].

The plant samples such as leaves, stem bark, fruits, and flowers were collected washed with distilled water thrice. From the flowers, petals and anthers and from fruit, fruit rind (ectoderm), and fruit pulp were separated for extraction and analysis. Afterward, the samples were dried on blotting sheet and then kept in hot air oven for drying at 60°C for 2-3 hrs. Dried samples were taken out and homogenized into a fine

powder using a mortar and pestle, stored in air tight container and were used for the extraction process (Fig. 1).

Extraction

Leaves, fruit rind, fruit pulp, stem bark, flower anther and flower petals powder was weighed, soaked, and dissolved in methanol (1:10), kept on shaker at 150 rpm for 48 hrs at 34°C and the filtered through filter paper (What Man No: 1) in a centrifuge tube. The filtrate was centrifuged at 5000 rpm for 10 minutes. The supernatant was taken in a fresh conical flask and kept on water bath for evaporation and used further analysis.

Qualitative analysis

Detection of flavonoids

Sodium hydroxide (NaOH) test

Methanolic extract of different parts of the *C. guianensis* was subjected to qualitative analysis to identify the presence of flavonoids content. The methanol (0.1 ml) extract of different parts was taken in test tubes, and then 0.1 ml of NaOH was added into the test tube. It gives yellowish color. After that 0.1 ml of diluted hydrochloric acid was added which changes yellow colored solution to colorless.

Ferric chloride test

The methanolic extract (0.1 ml) of different parts of plants were taken in test tubes, and then few drops of $FeCl_3$ were added, blue precipitate of solution was observed.

Quantification of total flavonoids

Methanolic extract 2 ml of different plant parts were incubated for 30 minutes at room temperature with 2 ml of methanol, 0.1 ml of aluminum chloride, and 0.1 ml of sodium acetate. After incubation, the intensity of the color formed was measured at 415 nm. A standard graph for quercetin (10-50 μ g) was plotted, from which the flavonoid content of the extract was determined [16,17]. The experiment was performed in triplicates.

HPLC analysis of flavonoids content of different parts of *C. guianensis*

The methanolic extract of *C. guianensis* anther, petals, fruit rind, and fruit pulp were prepared for HPLC by dissolving the 0.1 ml extract in 0.9 ml methanol. HPLC analysis was performed by Shimadzu HPLC system (Japan) with photodiode array detector (operated at 415 nm) and injection valve with 20 μ L sample loop. Compounds were separated on a 4.6x250 mm, 5 μ m pore size RP-C18G column protected by a guard column containing the same packing. The mobile phase was a gradient prepared from HPLC-grade water (Component a) and 100% methanol (Component b). Before use, the extract was filtered through 0.45 μ m membrane filter. A binary gradient was used as LC stop time 0-10 minutes, pump B concentration 70%. The flow rate was 1.0 ml minutes⁻¹. Data were integrated by Shimadzu lab solution software. Only four parts of *C. guianensis* was utilized for HPLC analysis in out of six.

Antibacterial assay

Microorganism used for antibacterial assay

The different bacterial strains were of American type culture collection were used for antibacterial analysis *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*.

Culture preparation for antibacterial assay

The different bacterial strains were grown on Luria-Bertani broth in a test tube and kept on shaker 37°C, 120 rpm overnight.

Agar well diffusion method

The standard method [18] with slight modification of Mueller-Hinton agar plates were prepared, and wells of 6 mm were cut and swabbed with different cultures and the cut wells were then filled with 50 μ l extract of different parts of the plants, and the plates were kept for incubation at 37°C for 24 hrs.

RESULTS AND DISCUSSION

Phytochemical screening

Qualitative analysis of flavonoids content of different parts of the plant was performed by NaOH and ferric chloride test. In the NaOH test, only the fruit rind, flower anthers, and flower petals show the presences of flavonoids while leaves did not showed any positive results or presence of flavonoids. Furthermore, analysis of flavonoids by ferric chloride showed the presence of flavonoids in all the plant parts (Table 1). Earlier [10] also confirms the presence of flavonoids in *C. guianensis* and suggested it is a warehouse of various active constituent.

Quantification of flavonoids content

The methanol extract of different plant parts was quantified for flavonoids content showed that leaves have the highest flavonoids content followed by flower anther, fruit pulp, fruit rind, and stem bark (Fig. 2). It is clearly shown that almost all the parts of the plant have medicinal value especially the leaves. Earlier phytochemical studies revealed the presence of triterpenoid glycoside, saponins [19], triterpenoid saponins [20], flavanol [21], glycosides, and indole constituents [22] in *C. guianensis*.

HPLC analysis of methanolic extract of *C. guianensis*

Qualitative analysis of presence of flavonoids content in *C. guianensis* flower petals, anthers, fruit pulp, and fruit rind was performed by HPLC and chromatographic profile compared with quercetin as reference compound for the presence of flavonoids (Fig. 3a-e). Analysis of HPLC spectrum of the methanolic extract of *C. guianensis* performed in the 415 nm wavelength, revealed the presence of peaks with more or less similar retention time for flower anther 3.103 and 3.204 (Fig. 3b), flower petal 2.839 (Fig. 3c), fruit pulp, and fruit rind with same reverse transcriptase (RT) 2.889. (Fig. 3d-e) chromatogram of different parts with variable RT showed that extract of different parts of the plant have the presence of not only single flavonoids although it contains a group of flavonoids.



Fig. 1: (a) *Couroupita guianensis* tree located in B S Abdur Rahman University, campus; different plant parts were collected from tree, viz, leaves, stem bark, (b) flower petals, anthers, (c) fruit rind (ectoderm) and fruit pulp

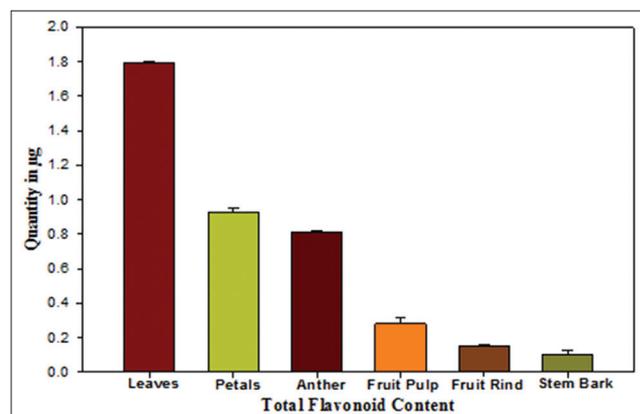


Fig. 2: Estimation of total flavonoids content of *Couroupita guianensis* leaves, petals, anthers, fruit pulp, fruit rind, and stem bark

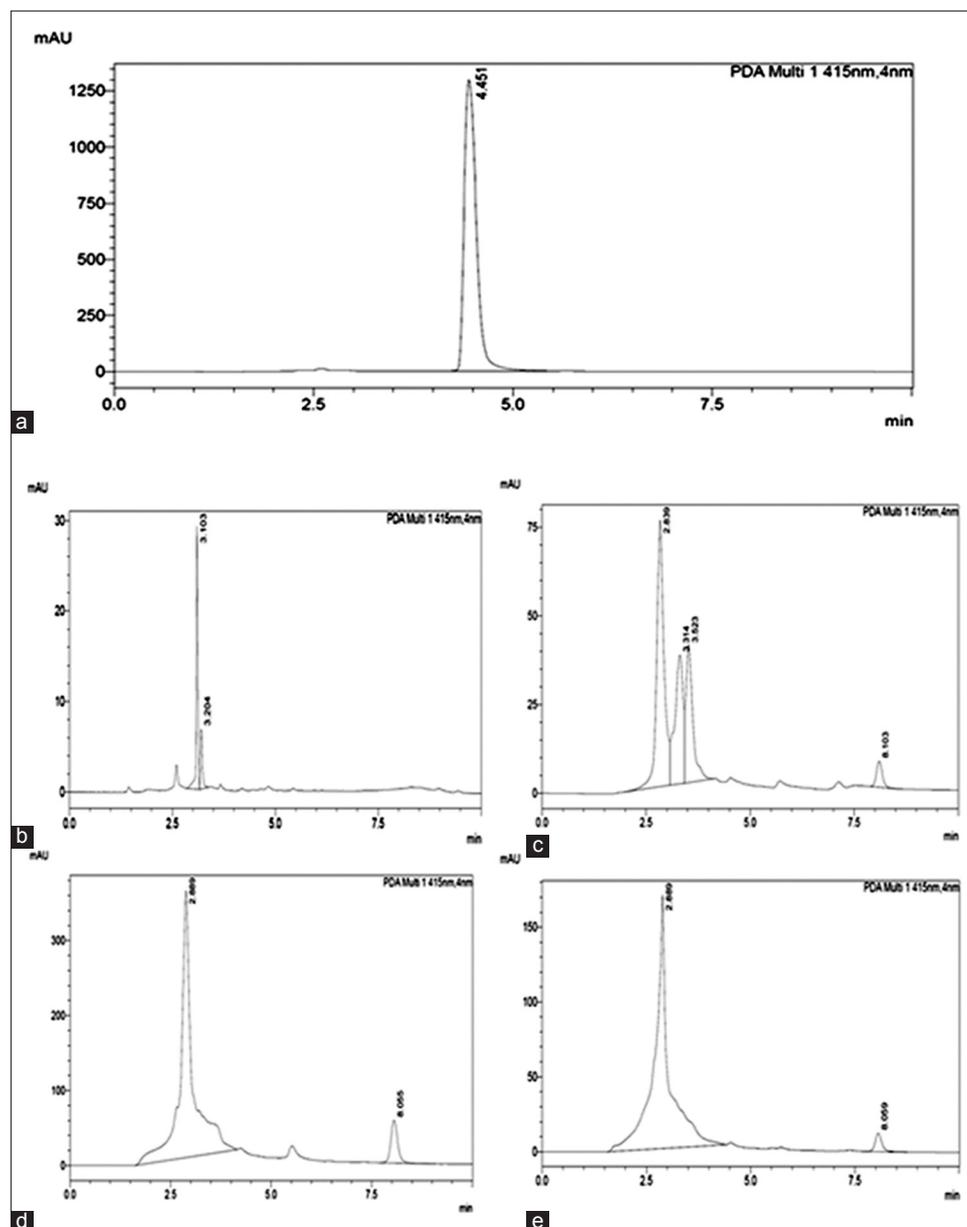


Fig. 3: High performance liquid chromatography chromatogram of (a) Quercetin as a standard of flavonoids, (b) flower anthers, (c) petals, (d) fruit pulp, and (e) rind

Test of antibacterial activity

The methanol extract of the *C. guianensis* leaves, petals, anther, fruit pulp, fruit rind, and stem bark was screened against six human pathogenic bacteria *E. coli*, *P. aeruginosa*, *P. mirabilis*, *A. baumannii*, *S. typhimurium*, and *K. pneumoniae* to insure the antibacterial activities by well diffusion method which showed valuable zone of inhibition (Fig. 4). The specific zone of inhibition against various types of pathogenic bacteria was shown in Table 2. The zone of inhibition against bacterial pathogens ranged between 08 and 22 mm in methanol extract. The maximum activity (22 mm) was recorded from fruit pulp extract of *C. guianensis* against *E. coli* and *K. pneumoniae* followed by *A. baumannii* 21 mm. The methanol extract of flower petals and anther showed zone of inhibition against all the six bacterium ranged between 12 and 18 mm. Leaves and stem bark showed inhibition only against the *P. mirabilis* 18 and 22 mm, respectively. Methanolic extract of fruit rind did not showed any zone of inhibition or antibacterial activity against any bacterial pathogen. Continuous and regular usage of antibiotics causes the antibiotic or multiple drug resistance [23] therefore plant extract may be an

alternative source of antibiotics to inhibit bacterial infection. The study coincides with earlier studies [24,25] and suggested that *C. guianensis* is a potential source of bacterial inhibition with various bioactive constituents and can be exploited to treatment against bacterial resistance.

CONCLUSION

Plants are the basic source of knowledge of modern medicine. Almost all the parts of the plant, namely leaves, flowers, fruit, bark, roots, stem, and seeds have various medicinal properties. The trend of using natural products has increased, and the active plant extracts were frequently screened for new drug discoveries. The present investigation reported, this plant is warehouse of chemodiversity, which will be useful in screening for medicine. Flavonoids are mainly present in all of the plant parts of *C. guianensis*. Further, research may be conduct to identify the different type of flavonoids present in the *C. guianensis* and novel flavonoids activity might be identify in other microorganisms.

Table 1: Qualitative screening of *C. guianensis* fruit, stem bark, flower anther, petals, and leaves extract

Phytochemicals	Test	Fruit rind	Fruit pulp	Stem bark	Flower anthers	Flower petals	Leaves
Flavonoids	NaOH	+	-	-	+	+	-
	FeCl ₃	+	+	+	+	+	+

(+) shows the presence of flavonoids and (-) shows the absence of flavonoids, *C. guianensis*: *Couroupita guianensis*, NaOH: Sodium hydroxide

Table 2: Different parts of *C. guianensis* showing the zone of inhibition against bacterial pathogen

S.N	Extract	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. typhimurium</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>
1	Fruit pulp	20 mm	22 mm	17 mm	22 mm	19 mm	21 mm
2	Fruit rind	-	-	-	-	-	-
3	Petals	13 mm	14 mm	12 mm	18 mm	15 mm	15 mm
4	Anthers	13 mm	13 mm	14 mm	12 mm	17 mm	14 mm
5	Leaves	-	-	-	18 mm	-	18 mm
6	Stem bark	-	-	-	22 mm	-	-
7	Ampicillin	19 mm	11 mm	23 mm	31 mm	8 mm	8 mm

E. coli: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *S. typhimurium*: *Salmonella typhimurium*, *P. mirabilis*: *Proteus mirabilis*, *A. baumannii*: *Acinetobacter baumannii*, *P. aeruginosa*: *Pseudomonas aeruginosa*

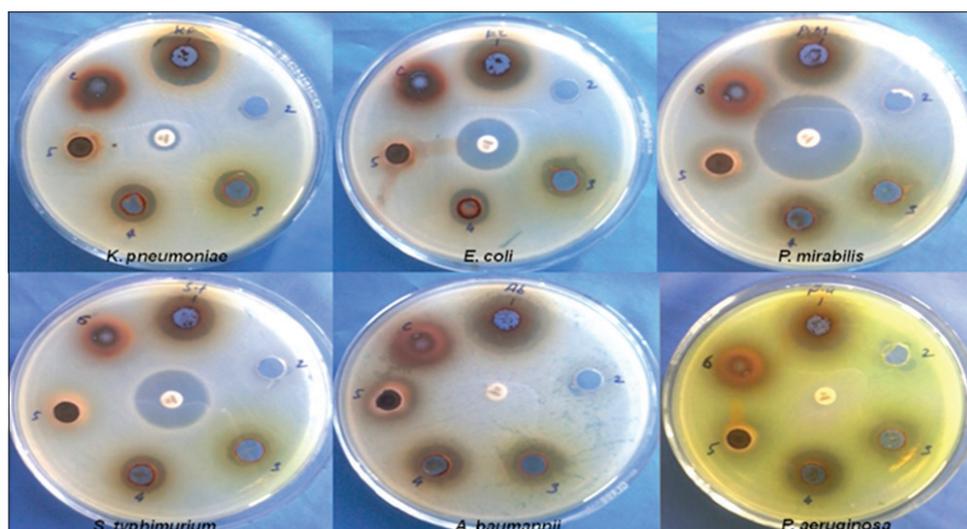


Fig. 4: Extract of different plant parts showing the zone of inhibition in *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*, (1) Fruit pulp, (2) fruit rind, (3) petals, (4) anthers, (5) leaves, (6) stem bark, and (7) antibiotic ampicillin

ACKNOWLEDGMENTS

Authors are thankful to B S Abdur Rahman University, Chennai for providing research fellowship to M S Khan for conducting the research.

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