

PRELIMINARY SCREENING OF PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT AND ANTIMICROBICIDAL ACTIVITIES IN THE METHANOLIC LEAF EXTRACT OF *COUROUPITA GUIANENSIS*

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ABSTRACT

Objective: The objective of the study was the preliminary screening of the methanolic leaf extract of *Couroupita guianensis* for various phytochemical constituents, its antioxidant, antibacterial, and antifungal activities.

Methods: Phytochemical screening of 18 qualitative, 6 quantitative constituents, antioxidant activity, alpha-amylase inhibitory activity, and alpha-glucosidase inhibitory activity of the methanolic extract of the leaf of *C. guianensis* was performed adopting the standard protocols. The disk diffusion methods were used for assessing the antibacterial and antifungal activities of the extract.

Results: The preliminary studies revealed the presence of alkaloids, saponin, flavonoids, phenol, tannin, and terpenoids in the methanolic extract of the leaf of *C. guianensis*. Potent antioxidant, free radical scavenging activity, and inhibitory activity against α -amylase and α -glucosidase activity of the methanolic extract were also evident.

Conclusions: The preliminary studies in the methanolic extract of the leaf of *C. guianensis* are suggestive of the therapeutic potentials of the methanolic extract of leaves of *C. guianensis*.

Keywords: *C. guianensis*, methanolic extract, antioxidant, antimicrobial

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INTRODUCTION

Diabetic mellitus is a chronic metabolic disorder reported to be one of the leading causes of death. 90–95% of diabetes is of type 2 diabetes mellitus, a heterogeneous disorder due to insulin resistance or due to faulty insulin secretion [1]. Survey of literature reveals an ocean of information on the medications available for the treatment of diabetes, namely, anti-diabetic drugs and as well as human/pork/beef insulin all of which decrease blood glucose level [2,3]. On the other hand, serious side effects such as gastrointestinal flatulence, diarrhea, and abdominal pain caused by these anti-diabetic drugs cannot be ignored [4]. Hence, the need for an alternate natural remedy for diabetes is the need of the hour. WHO has reported more than 21,000 plants used for medicinal purposes, of which about 300 plants used for the treatment of diabetes [3].

A number of authors have documented the anti-inflammatory, anti-helminthic, and antioxidant activity of parts of *Couroupita guianensis* such as leaf, fruit, and root. They have affirmed the presence of many phytochemical compounds such as ketosteroids, oils, courouptine, and indirubin [5-7]. A preliminary screening of phytochemical constituents, antioxidant, antibacterial, and antifungal activities in the methanolic leaf extract of *C. guianensis* was undertaken in this research work.

MATERIALS AND METHODS

Collection of Plant Material

Leaves of *C. guianensis* were collected in the month of November from Kavandampatti at Karur district in Tamil Nadu, India. The plant authentication (PT 001) was confirmed by Rapinat herbarium at St. Joseph College, Tiruchirappalli, Tamil Nadu.

Preparation of Extract

C. guianensis leaves were washed in running tap water, cut into small pieces, shade dried for a week, thence grinded to form a uniform-

sized powder, and sieved in mesh size 30. One hundred grams of the dried powder were soaked in 1 liter of methanol for 10 h in a Soxhlet extractor. The extract was filtered using Whatman filter paper, added DMSO, and used for further analysis.

Phytochemical Screening

Standard procedures were used for the screening of phytochemical constituents from the methanolic leaf extracts of *C. guianensis* [8-10]. Eighteen phytochemical constituents were screened qualitatively, namely, tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, anthocyanins, leucoanthocyanin, emodin, anthraquinone, proteins, coumarins, phenols, glycosides, alkaloids, xanthoproteins, and carbohydrates.

Six phytochemical constituents, namely, flavonoid, tannin, saponin, alkaloid, phenol, and terpenoids, were assessed quantitatively.

Antioxidant Activity

The ability of the methanolic leaf extracts of *C. guianensis* to scavenge the free radical DDPH was determined to assess the antioxidant activity as adopted by Braca *et al.* [11].

Alpha Amylase and Alpha-glucosidase Inhibitory Activity

The alpha-amylase inhibitory activity of the methanolic leaf extracts of *C. guianensis* was estimated using a modified procedure adopted by McCue and Shetty [12]. The alpha-glucosidase activity was computed adopting the method described by Kim *et al.* [13].

Antimicrobial Activity

Disk diffusion method was used to determine the antibacterial and antifungal activities of crude methanolic leaf extracts of *C. guianensis*. Mueller–Hinton agar was used for the bacterial culture and Sabouraud's dextrose agar was used for the culture of fungal organisms. Sterile disk

of 6 mm width was used for preparing different concentrations of crude methanolic leaf extracts of *C. guianensis*. The Petri plates were inoculated with the test organisms, disks implanted and incubated. The zone of inhibition was measured around each well after 24 h at 37°C. Disk impregnated in DMSO was used as control.

Statistical Analysis

SPSS Windows Students version software was used to analyze the results of the quantitative data, which is expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical Screening

Qualitative assay for the various phytochemical compounds revealed the presence of 14 constituents, namely, tannins, saponins, flavonoids, steroids, terpenoids, leucoanthocyanin, anthocyanin, coumarins, glycosides, phenol, alkaloids, xanthoproteins, emodin, and carbohydrates (Table 1). On the other hand, compounds such as cardiac glycosides, phlobatannins, anthraquinone, and proteins were not reported in our experiments. A number of researchers have also reported the presence of a wide range of one or more of these compounds in solvent extracts from different parts of *Stevia rebaudiana* [14]; *Gymnema montanum* [15]; aqueous root bark extract of *Blighia sapida* [16]; and in leaves of *C. guianensis* [17]. Anticancer potentials in the polysaccharides isolated from the methanolic extract of *Tinospora cordifolia* stem bark has also been established [18]. Furthermore, many authors have also affirmed the presence of many phytochemical compounds such as ketosteroids, oils, courouptine, and indirubin in various parts of *C. guianensis* [5,7,19] Thus, presence of the various phytochemical compounds in *C. guianensis* can thus be validated.

The amount of flavonoid, tannin, saponin, alkaloid, phenol, and terpenoid estimated from the methanolic extracts of leaves of *C. guianensis* is presented in Table 2. Least concentration of $1.1 \pm 0.06 \times 10^2$ mg/100 g of phenol and a maximum quantity of flavonoid with $30.4 \pm 1.52 \times 10^2$ mg/g was evident. The quantity of terpenoids, alkaloids, tannins, and saponins were found to show an ascending trend with $1.7 \pm 0.09 \times 10^2$, $2.4 \pm 0.12 \times 10^2$, $10.4 \pm 0.52 \times 10^2$, and $18.1 \pm 0.91 \times 10^2$ mg/g, respectively. Many authors have also affirmed the presence of these constituents in *C. guianensis* [20,21].

Antioxidant Activity

The percentage inhibition of the activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed as an assay of the antioxidant activity of the methanolic leaf extract of *C. guianensis*. The methanolic leaf extract of *C. guianensis* revealed 72.36% of inhibition at 100 µg/ml concentration. The percentage antioxidant activity at 10 µg/ml was 48%, while the same at 40, 60, and 80 µg/ml was 55%, 59%, and 61%, respectively. Researchers relate the high phenolic content of the extract to the increased antioxidant activity. Studies on an ethanol extract of leaves of *C. guianensis* revealed effective concentration-dependent scavenging activity on DPPH [22]. Similar results have been reported by Vetrichelvan *et al.*, Bender *et al.*, Sincy *et al.*, Hanamura *et al.* [23-26]. Powerful antioxidant activities have been reported in the methanolic leaf extracts of *Dypsis leptocheilos* [27] and in the aqueous extract of *Oldenlandia corymbosa* [28] also. A number of authors evaluated the anti-oxidative and anti-diabetic properties of green tea mixture and leaves of *Pluchea* plant [29-32]. They have reported the presence of α glucosidase inhibitory activity and α amylase inhibitory activity. Survey of literature revealed a number of potentials catechins present in green tea that exhibited antioxidant and prebiotic activities [30], antidiabetic [31], and analgesic effects [33].

Alpha Amylase and Alpha-glucosidase Inhibitory Activity

Methanolic leaf extract of *C. guianensis* at various concentrations (20, 40, 60, 80, and 100 mg/ml) showed efficient α-amylase inhibitory activity. Researchers have suggested the α-amylase inhibitory activity to be associated with the presence of saponin, stigmasterol, and

Table 1: Phytochemical constituents in methanolic extract of leaves of *C. guianensis*

Phytochemical constituents	Methanolic extract of leaves of <i>C. guianensis</i>
Tannin	+++
Phlobatannins	-
Saponin	+++
Flavonoid	+
Steroids	+++
Terpenoids	+++
Cardiac glycosides	-
Leucoanthocyanin	+++
Anthocyanin	+++
Anthraquinone	-
Protein	-
Coumarins	+
Glycosides	+++
Phenol	+++
Alkaloids	+++
Xanthoproteins	+
Emodin	+
Carbohydrates	+++

C. guianensis: *Couroupita guianensis*

Table 2: Quantitative analysis of methanolic extract of leaves of *C. guianensis*

Phytochemical constituents	Mean±SD (mg/100 g)
Flavonoid	$30.4 \pm 1.52 \times 10^2$
Tannin	$10.4 \pm 0.52 \times 10^2$
Saponin	$18.1 \pm 0.91 \times 10^2$
Alkaloid	$2.4 \pm 0.12 \times 10^2$
Phenol	$1.1 \pm 0.06 \times 10^2$
Terpenoid	$1.7 \pm 0.09 \times 10^2$

SD: Standard deviation; *C. guianensis*: *Couroupita guianensis*

lupol [23-25,34,35]. The percentage of alpha-glucosidase inhibitory activity in the methanolic leaf extracts at a concentration between 20 and 100 µg/ml revealed an effect between 33.33% and 80.70%. Researchers have affirmed the methanol extract of *Tinospora sinensis* to possess maximum α-amylase inhibitory activity when compared to their ethanolic extract [26]. A number of authors have suggested that polyphenol and flavonoid have the ability to inhibit intestinal alpha-glucosidase and pancreatic alpha-amylase [36,37]. Bioactive constituents such as tannins, phenols, alkaloids, saponins, steroids, and flavonoids isolated from the extract of *Croton bonplandianum* have been reported to have efficient α-amylase inhibitory activity [22]. The presence of antioxidant and α-amylase inhibitory activity in *Phyllanthus indofischeri* extract is suggestive of their use as a natural antioxidant and hypoglycemic agent [38].

Antimicrobial Activity

Antibacterial activity of the crude methanolic leaf extract of *C. guianensis* at a concentration of 100 µg/ml produced zone of inhibitions of 7 mm, 6 mm, and 7 mm against *Escherichia coli*, *Staphylococcus aureus*, and *Enterobacter aerogenes*, respectively (Plate 1). The methanolic extract exhibited inhibitory actions against pathogens at concentrations of 60, 80, and 100 mg/ml than at lower concentrations (Table 3). As the concentration of extracts increased from 20 to 100 mg/ml, the inhibitory actions of the plant extracts increased toward all the strains used in this test. However, a significant antibacterial effect could not be attributed to the extract used in the present study.

The antifungal susceptibility test of the plant extracts, against the test organisms, indicated that the methanolic extracts posed no impact on *Candida albicans* and *Candida tropicalis* (Table 4). There was no zone of inhibition developed for the two tested organisms against

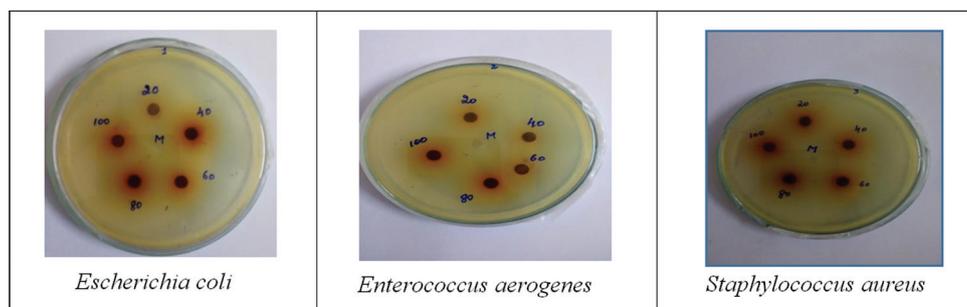


Plate 1: Photographic image of the antibacterial activity of the methanolic leaf extract of *Couroupita guianensis* against the three chosen bacterial strains

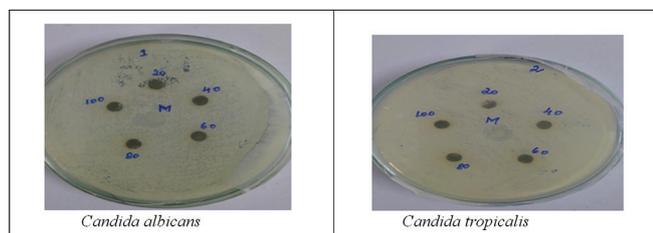


Plate 2: Photographic image of the antifungal activity of the methanolic leaf extract of *Couroupita guianensis* against the two chosen fungal strains

Table 3: Antibacterial activity of leaves of *C. guianensis*

Concentration of extract (µg/ml)	Diameter of zone of inhibition (mm)		
	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)	<i>E. aerogenes</i> (mm)
20	0	2	5
40	4	3	6
60	5	4	7
80	6	5	6
100	7	6	7
10 µl/disc	0	0	0

C. guianensis: *Couroupita guianensis*; *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*; *E. aerogenes*: *Enterobacter aerogenes*

Table 4: Antifungal activity of methanolic leaf extracts of *C. guianensis*

Concentrations (µg/ml)	Diameter of the zone of inhibition (mm)	
	<i>Candida albicans</i>	<i>Candida tropicalis</i>
20	0	0
40	0	0
60	0	0
80	0	0
100	0	0
10 µl/disc	0	2

C. guianensis: *Couroupita guianensis*

the methanolic extracts of *C. guianensis* (Plate 2). Survey of literature revealed that flavonoids possess antioxidant properties and give protection against allergies, ulcers, inflammation, platelet aggregation, microbes, hepatotoxins tumors, and viruses [19]. In the present study, significant antifungal activity was not established.

CONCLUSIONS

Methanolic leaf extracts of *C. guianensis* showed significant enzyme inhibitory activity, antioxidant, and antibacterial activities. However, further research on the isolation of the active phytoconstituents with

therapeutic activity and clinical study for the evaluation of safety and efficacy of the drug needs to be assessed.

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AUTHORS' CONTRIBUTIONS

Research guidance – Dr. Sujatha Ilangovan, experimental work and manuscript writing – P. Thavasumani, and editing and proof correction – Dr. Sujatha Ilangovan.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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