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Antioxidant, phytochemical screening and antimicrobial activity of *Couroupita Guianensis* flower extract

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ABSTRACT

The present study was aimed to evaluate the antioxidant potential and antibacterial activity of methanol extract of flowers of Couroupita guianensis. Flowers of Couroupita guianensis were collected and identified by a botanist. A Methanol extract of the dry pulverized flowers of Couroupita guianensis was obtained by the Soxhlet cold extraction method. The methanol flower extract was subjected to a preliminary phytochemical screening by standard methods. In vitro antioxidant activity was assessed by 1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay and measuring hydrogen peroxide radical scavenging activity. Ascorbic acid was used as the standard antioxidant for comparison. The antibacterial activity of flowers was investigated by well diffusion method. Phytochemical analysis of the extract showed the presence of major classes of phytochemicals alkaloids, phenolic compounds such as flavonoids, tannins and saponins. Couroupita guianensis methanolic extract showed the highest reducing capacity. The antibacterial activity of plant extract was found significant. The results of our study suggest that flowers of Couroupita guianensis possess potent antibacterial activity and are a good source of natural antioxidants. Further study is required to identify the chemical compounds responsible for their antibacterial activity.

Keywords: *Couroupita guianensis.* Antioxident and Antibacterial

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INTRODUCTION

Couroupita guianensis (Aubl) belongs to the family Lecythidaceae, commonly known as cannon ball tree. The plant has great therapeutic implication in Indian system of medicine and described. It is used extensively as an ingredient in many preparations which cure gastritis, scabies, bleeding piles, dysentery, scorpion poison and many (Alagesaboopathi *et al.*,2013). It has rubefacint and anti-rheumatic properties used in Ayurveda concepts, cold relief balm and fruit pulp are used to cure headache, the flowers are used to cure cold, intestinal gas formation and stomach ache, and also for treating diarrhea, and when dried and powdered, used as a snuff (Gousia *et al.*,2012). The fragrance of flowers is used for curing asthma and the shell of the fruit is used as a utensil. Over the past few years, there has been an exponential growth in study of pharmacological properties of this plant. Antioxidant components are micro constituents that inhibit lipid oxidation by inhibiting the initiation or propagation of oxidizing chain reactions, and are also involved in scavenging of free radicals. Clinical approaches of antioxidants increased multi fold during the recent time for the management and therapeutic implication of neurodegenerative disorders, aging, and chronic degenerative diseases (Harborne *et al*., 1998). In view of the above, we designed the study to evaluate the antioxidant potential and antibacterial effect of *Couroupita guianensis.*

MATERIALS AND METHODS

Plant Material:
Couroupita guianensis flowers were collected during the month of August from Thiruvalam temple, Vellore Dist., Tamilnadu, India. The plant material was taxonomically identified and authenticated by Dr P.Jayaraman, Professor in Botany, Madras University.

Extraction:
The dried flower (250g) of C. *guianensis* (sample CGA) was extracted with methanol (500ml) using soxhelet apparatus. Then the methanol extract was concentrated, and weighed (10g). The dry extract was stored at 4^0C until used.

Qualitative phytochemical screening of Couroupita guianensis flower extract: Phytochemical screening of methanol extract (Manimegalai *et al*., 2012) The methanol extract of *Couroupita guianensis* flowers was subjected to qualitative phytochemical analysis for the presence of various classes of active chemical constituents such as reducing sugar, proteins, alkaloids, Phenols, tannins, saponins, glycosides, flavonoids, terpenes and steroids etc. using standard procedures (Hossain *et al.*, 2013)

Determination of antioxidant activity by DPPH-scavenging assay:
The free radical scavenging activity of the flower extract of *Couroupita guianensis* and of standard solution (ascorbic acid) was investigated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method as reported in the literature (Asadujjaman *et al*., 2013). The assay mixture contained 2 ml of 1.0 mmol/L DPPH radical solution prepared in methanol and 1 ml of standard or extract solution of different concentrations (50-500 µg/ml). The solution was rapidly mixed and incubated in the dark at 37 ^{0}C. For 20 min. The decrease in absorbance of each solution was measured at 517 nm using UV/V is spectrophotomer. Ascorbic acid, a well-known antioxidant was used as positive control while the DPPH radical solution with 1 ml methanol was taken as blank. The percentage of radical scavenging (%) was calculated by the following formula:

DPPH scavenging activity (%) = $[A_0 – A_1/ A_1] \times 100$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample. The results are averages of five measurements. Free radical scavenging activity is expressed as the percentage of DPPH Decrease (Gawron-Gzella *et al*., 2012).

Determination of antioxidant activity by hydrogen peroxide scavenging assay:
Hydrogen peroxide is generated in vivo by several oxidase enzymes. There is increasing evidence that hydrogen peroxide is scavenged, either directly or indirectly via its reduction product hydroxyl radical (OH•). In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm (Keser *et al.,* 2012). Different concentrations of *Couroupita guianensis* were prepared in methanol in the concentration range 50-500 µg /ml. Standard solution of ascorbic acid was prepared in the same concentration range. 1ml of various concentrations of sample and standard were mixed with 2ml of 20 mM hydrogen peroxide in phosphate buffer saline (pH7.5). After 10 minutes absorbance was measured at 230 nm against phosphate buffer saline (pH 7.5) as blank. The experiment was performed in triplicate. Scavenging activity was determined by comparing % inhibition with that of control (100%) containing only H_2O_2 and solvent. The data was expressed as % inhibition.

Hydrogen peroxide scavenging activity (%) = $[A_0 – A_1/ A_1] \times 100$

A panel of Microorganisms:
A board of organisms comprising *Escherchia coli* (MTCC 1698), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 9878) *and Salmonella enterica* (MTCC 9844) These microbes was selected to test the *Couroupita guianensis* extracts ability to inhibit the growth.. Prior to sensitivity testing, each of the bacteria strains were cultured onto nutrient agar plates and incubated for 18 to 24 h at 37°C to obtain colonies. After overnight incubation, colonies were selected with a sterile disposable inoculating loop and transferred to a glass tube of sterile physiological saline and vortex thoroughly. Each bacterial suspension turbidity is then compared to that of the 0.5 McFarland standard solution (containing about 1.5 × 108 CFU/ml) (Caroling *et al*., 2013).

Antibacterial Activity:
Antimicrobial susceptibility testing was done using the well-diffusion method according to the standard (Priya *et al*., 2012). The plant extracts were tested on Mueller Hinton plates to detect the presence of antibacterial activity (Sivakumar *et al*., 2012). Prior to streaking the plates with bacteria, 5 mm diameter wells were punched into the medium using a sterile borer. All plates were inoculated with the test bacterium which has been previously adjusted to the 0.5 McFarland standard solution; a sterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level removing excess inoculum. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculum with a final swab around the rim. The plates are allowed 3 to 5 min to dry the excess moisture. Different concentration of aliquots of each test extract was dispensed into each well after the inoculation of the plates with

bacteria. The wells were also arranged in a triangle formation 2 inches apart. The same extract was used on each plate, with a total of three plates used for each extract for selecting bacterium. Sparfloxacin was used as positive control and DMSO was used as negative control (C) For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The plates are sealed with parafilm, labelled, and placed in an incubator set to 37°C. After 24 hours of incubation, each plate was examined for inhibition zones. A ruler was used to measure the inhibition zones in millimeters. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments (Voukeng *et al*.,2012).

RESULTS AND DISCUSSION

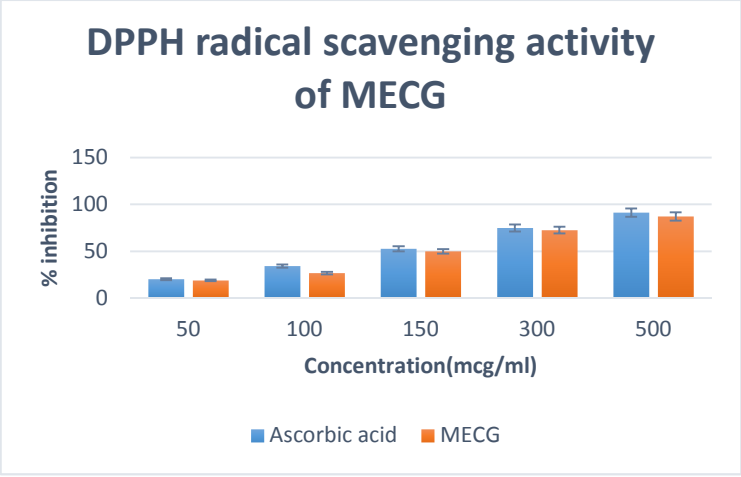
Phytochemical screening:
Phytochemical screening of the leaf extracts of *Couroupita guianensis* confirmed the presence of alkaloids, phenols, flavonoids and reducing sugars (Table 1).

TABLE 1. Phytochemical screening of the leaf extracts of *Couroupita Guianensis*

S.NO	Test for	Result
1	Carbohydrates	+
2	Proteins	-
3	Alkaloids	+
4	Phenols	+
5	Tannins	+
6	Saponins	+
7	Glycosides	+
8	Flavonoids	+
9	Terpenes	+
10	Steroids	-

In vitro antioxidant activity:
Free radical scavenging ability on 2, 2-diphenyl-2picrylhydrazyl (DPPH):
The radical-scavenging activity of the methanol extracts of *Couroupita guianensis* was estimated by comparing the percentage inhibition of formation of DPPH radicals with that of vitamin C. The DPPH radical scavenging activity of methanol extracts increased with increasing the concentration. Our results were radical scavenging activity of 18.96, 26.71, 49.82, 72.49 and 87.21% in the methanolic extract of *Couroupita guianensis* at a concentration of 50, 100,150,300and 500 µg/ml, respectively. Natural antioxidants, those are present in medicinal plants, are responsible for inhibiting the harmful consequences of oxidative stress. Many plant extract exhibit efficient antioxidant properties due to their phytoconstituents, including phenolic. This method has been extensively used for screening antioxidants, such as polyphenols. The antioxidant effectiveness in natural sources has been reported to be mostly due to phenolic compound (Table 2)

Figure 1 In-vitro antioxidant activity by DPPH scavenging method



Hydrogen peroxide radical scavenging activity:
Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with and Fe^{2+}possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its oxide effects (Rahman et

al., 2012). From the results, it appeared that the H_2O_2 scavenging activity of the plant extract is significant compared to that of the standard ascorbic acid. Figure 2 shows the hydrogen peroxide scavenging activity of *Couroupita guianensis* in comparison with Ascorbic acid standard (Table 3).

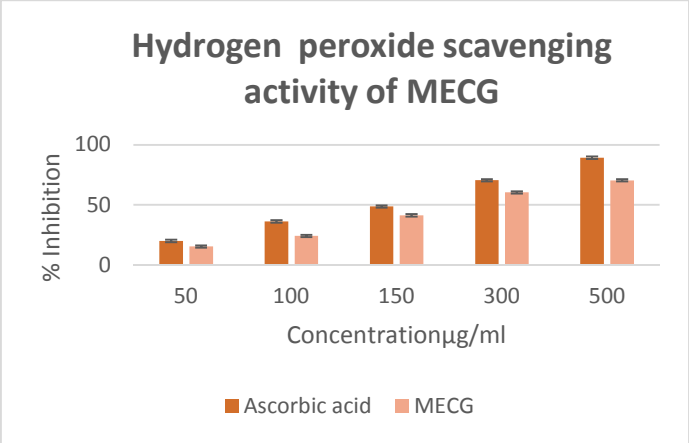
TABLE 2: In-vitro antioxidant activity by DPPH scavenging method

Concentration in µg/ml	Standard (Ascorbic acid)	MECG
50	20.90 ± 0.14	18.96 ± 0.21
100	34.21 ± 0.23	26.71 ± 0.13
150	52.62 ± 0.13	49.82 ± 0.24
300	74.80 ± 0.12	72.49 ± 0.12
500	91.27 ± 0.18	87.21 ± 0.21

TABLE 3: In-vitro antioxidant activity by hydrogen peroxide radical scavenging method

Concentration in µg/ml	Standard (Ascorbic acid)	MECG
50	19.90 ± 0.13	15.32 ± 0.13
100	36.21 ± 0.23	24.12 ± 0.52
150	48.64 ± 0.13	41.22 ± 0.24
300	70.44 ± 0.15	60.31 ± 0.11
500	89.27 ± 0.12	70.41 ± 0.32

Figure 2 In-vitro antioxidant activity by Hydrogen peroxide scavenging method



Antimicrobial activity:

The antimicrobial activity of the extracts of *Couroupita guianensis* was studied in different concentrations (100,200, and 300 µg/ml) against four pathogenic bacterial strains, (*Escherichia coli* MTCC 1698, Staphylococcus aureus MTCC 3160, *Bacillus subtilis* MTCC 9878, *Salmonella enterica* MTCC 9844). These strains have been selected for the basis of its application purpose of further formulation study. Antibacterial potential of extracts was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Table 4.

TABLE 4. Antibacterial activity of the flower extract of *Couroupita Guianensis* by agar well diffusion method

S.NO	Type Of Strain	Zone of inhibition (mm)			Standard (mm)
		(100µg/ml)	(200µg/ml)	(300µg/ml)	
1	*Escherchia coli*	14.3±0.24	17.1±0.32	22.4±0.28	26
2	*Staphylococcus aureus*	15.3±0.24	18.2±0.32	23.2±0.52	26
3	*Bacillus subtilis*	16.2±0.24	17.4±0.34	24.3±0.48	26
4	*Salmonella enterica*	15.6±0.23	19.3±0.42	24.1±0.28	26

The antibacterial activities of the extracts increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for bacterial activity, *Bacillus subtilis and Salmonella enterica* were more sensitive as compared with *E. coli* and *Staphylococcus aureus.* The growth inhibition zone measured ranged from 14 to 24 mm for all the sensitive bacteria (Image 1). The results show that the extracts of *Couroupita guianensis* were found to be more effective against all the microbes tested.

FIGURE 3 Antibacterial activity of the flower extract of *Couroupita Guianensis* by agar well diffusion method

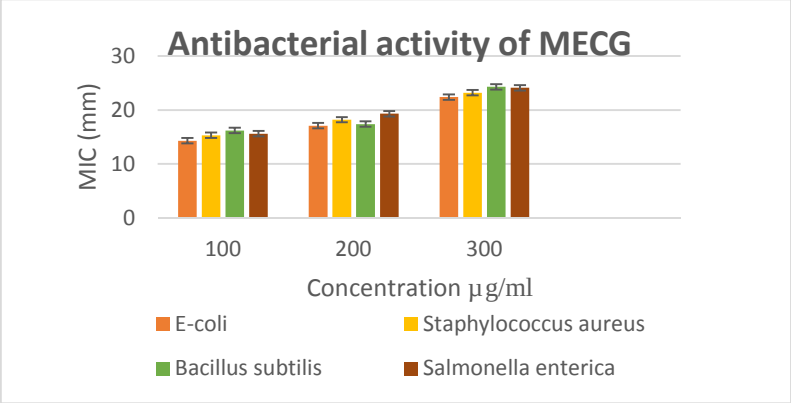
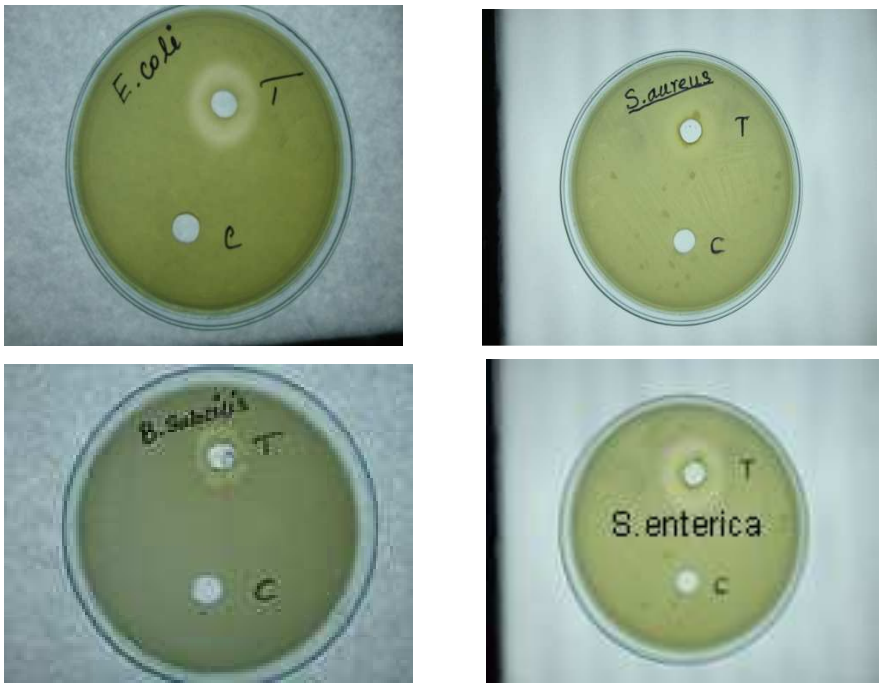


IMAGE 1: Antibacterial activity of the flower extract of *Couroupita Guianensis* by agar well diffusion method



CONCLUSION

The present study concludes that flowers of *Couroupita guianensis* contain high antioxidant and antibacterial property. The presence of phytochemicals such as reducing sugar, proteins, alkaloids, phenols, tannins, saponins, glycosides, flavonoids, terpenes and steroids. The plant extract certainly is effective in the management of the disease caused by these organisms. There is needs for further investigation of the plant in order to identify and isolate its active anti-bacterial and antioxidant principle(s)

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