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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF FLOWERS AND LEAVES OF *COUROUPITA GUIANENSIS AUBL*.

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ABSTRACT

Free radicals have been regarded as a fundamental cause of different kinds of diseases. They cause biochemical damage in cells and tissues, which result in several diseases such as arteriosclerosis, ischemia-reperfusion injury, liver disease, diabetes mellitus, inflammation, renal failure, aging, cancer, etc. Compounds that can scavenge free radicals are thus effective in ameliorating the progress of these related diseases. *Couroupita guianensis* known locally as 'Nagamalli flowers or Mallikarjuna ' and belonging to the family Lecythidaceae is a common plant in India And South America, profoundly used in Ayurvedic medicine for its antibiotic, antifungal, antiseptic and analgesic properties. It is also used for the treatment of gastritis, scabies, bleeding piles, dysentery and scorpion poison. The objective of the present study was designed to investigate the antioxidant efficacy of ethanolic extract of *Couroupita guianensis* leaves and flower (CGLE & CGFE). Invitro antioxidant activity such as Reducing power ability, NBT reduction assay, Deoxyribose degradation assay was performed and was concluded that the ethanolic extract of leaves and flowers of *Couroupita guianensis* showed significant antioxidant activity.

Keywords: *Couroupita guianensis*, Antioxidant activity, flavonoids, polyphenols.

INTRODUCTION

Free radicals have been regarded as a fundamental cause of different kinds of diseases. They cause biochemical damage in cells and tissues, which result in several diseases such as arteriosclerosis, ischemia reperfusion injury, liver disease, diabetes mellitus, inflammation, renal failure, aging, cancer, *etc.* [1]. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate various types of degenerative diseases. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties. Plants are potent biochemical factories and have been components of phytomedicine since times immemorial. *Couroupita guianensis* Aubl also called cannon ball tree belongs to family *Lecythidaceae* [2]. In *Ayurveda*, it is called as ayahuma, it is used extensively as an ingredient in the many preparations which cure gastritis, scabies, bleeding piles, dysentery, scorpion poison and many [2, 3]. It has rubefacint and anti rhumatic properties used in Ayurvedic concepts, cold relief balm. Fruit pulp is used to cure headache. In flok medicine, the flowers are used to cure cold, intestinal gas formation and stomach ache, and also for treating diarrhoea, and when dried and powdered, used as a snuff. The fragrance of flowers is used for curing asthma. The shell of the fruit is used as a utensil. The flowers of *C. guianensis* showed analgesic and anti-inflammatory activity and immunomodulatory activity [4, 5].

OBJECTIVE

Couroupita guianensis, also called as Cannonball Tree, is a native of India, Sri Lanka or South America. The tree is deciduous and large, have been reported for various pharmacological activities to treat diseases like gastritis, scabies, bleeding piles, dysentery, scorpion poison An attempt was made to correlate these activities with generation of free radicals and to evaluate the selected plant for its antioxidant potential [6].

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EXPERIMENTAL METHODS

Fresh leaves and flowers of the plant were collected from Bapatla, Guntur district. (A.P), India during the month of August 2011. The plant was identified and authenticated by the Dr.M.Raghuram, Assistant professor, Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur. . (A.P), India.

Extract preparation

The leaves and flowers of *Couroupita guianensis* were collected, thoroughly dried under shade and powdered mechanically and sieved through No.20 mesh sieve [7]. The finely powdered leaves and flowers were kept in an airtight container until the time of use.

Extraction procedure for leaf

The extraction was carried out by cold maceration and mechanical shaking method. The solvent used was 95% ethanol. About 60 g of powder was soaked with 600ml of 95% ethanol for 12 h and then macerated at room temperature using a mechanical shaker for 4 h. The extract was filtered off and the marc was again soaked with the same volume of 95% ethanol for 12 h and then further extracted for 4h and filtered. The filtrates were then combined concentrated under reduced pressure and evaporated at 400C. The percentage yield of the *Couroupita guianensis* leaf extract (CGLE) was 26.4%.

Extraction procedure for flower

The extraction was carried out by continuous hot percolation method using Soxlet apparatus. The solvent used was 95% ethanol. About 50 g of powder was extracted with 400 ml of solvent. The extract was concentrated to dryness under controlled temperature between 40-50ºC. The percentage yield of the *Couroupita guianensis* flower extract (CGFE) was 19.2% [8].

Phytochemical Screening of the Extract

Chemical tests were carried out for the ethanol extracts of flower and leaves extracts of *Couroupita guianensis* for the presence of phytochemical constituents.

***In Vitro* Methods Employed In the Study**

Reducing power ability [9]

Reducing power ability was measured by mixing 1.0 ml fractions of various concentration prepared with distilled water to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50ºC for 30 min. After that 2.5 ml of trichloroacetic acid (10%) were added to the mixture and centrifuged for 10 min at 3000 rpm, 2.5 ml from the upper part were diluted with 2.5 ml water and shaken with 0.5 ml fresh 0.1% ferric chloride. The absorbance was measured at 700 nm using UV-spectrophotometer. The reference solution was prepared as above, but contained water instead of the samples. Increased absorbance of the reaction mixture indicates increased reducing power. All experiments were done in triplicate using butylated hydroxyltoluene (BHT) as positive control.

Superoxide anion scavenging (NBT reduction) assay

A reaction mixture with a final volume of 3 ml per tube was prepared with 1.4 ml of 50 mM KH2PO4-KOH, pH 7.4 containing 1 mM EDTA, 0.5 ml of 100 µM hypoxanthine, 0.5 ml of 100 µM NBT. The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100 µl of phosphate buffer and 0.5 ml of test extract fractions in saline. The xanthine oxidase was added last. The subsequent rate of NBT reduction was determined on the basis of spectrophotometric determinations of absorbance at 560 nm. Ascorbic acid was used as standard. The results are expressed as the percentage inhibition of NBT reduction rate with respect to the reaction mixture without test compound (saline only).

Hydroxyl radical scavenging (Deoxyribose degradation) assay [10]

The decomposing effect of extract on hydroxyl radicals was determined by the assay of malondialdehyde chromogen formation due to 2-deoxy 2-ribose degradation. The assay mixture contained in a final volume of 1ml: 100 µl of 28 mM 2-deoxy 2-ribose dissolved in phosphate buffer, pH 7.4, 500 µl of the plant extract fractions of various concentrations in buffer, 200 µl of 200 mM ferric chloride (1:1 v/v) and 1.04 mM EDTA and 100 µl of 1.0 mM hydrogen peroxide and 100 µl of 1.0 µM ascorbic acid. After incubation of the test sample at 37^{o}C for 1 h the extent of free radical damage imposed on the substrate deoxyribose was measured using thiobarbituric acid (TBA) test. Percentage inhibition of deoxyribose degradation was calculated. Quercetin was used as standard.

Statistical analysis

Results were statistically evaluated by analysis of variance (ANOVA) followed by Dunnets test, $P < 0.05$ was considered to be statistically significant.

Results and discussion:

Phytochemical screening

Phytochemical screening of the ethanolic extracts of CGLE and CGFE revealed the presence of alkaloids, flavonoids, glycosides, and phenols.

***In vitro* antioxidant activity**

Reducing power ability

Table 2 shows the reductive capabilities of CGLE and CGFE when compared to the standard butylated hydroxy toluene (BHT). The increase in absorbance of the reaction mixture containing the extract showed increased reducing power with increase in concentration. The reducing power increased significantly ($P<0.01$) with increasing amounts of the extract. However, the activity of the CGLE and CGFE was less than the standard.

Superoxide radical scavenging activity

The CGLE and CGFE were found to be a scavenger of superoxide anion generated in xanthine oxidase-NBT systems (Table 3). The extract showed significant (P<0.01) superoxide inhibiting activity at a concentrations of 25-400 µg/ml. The IC_{50} of the CGLE and CGFE was found to be 188.39 ± 0.42 and 318.13 ± 0.21 respectively where as the IC_{50} of the standard ascorbic acid is 92.46µg/ml.

Hydroxyl radical scavenging (Deoxyribose degradation) assay

The degradation of deoxyribose by Fe^{3+}-ascorbate–EDTA-H_2O_2 system was markedly decreased by CGLE and CGFE indicating the significant (P<0.01) hydroxyl radical scavenging activity. The IC_{50} of quercetin was 24.5µg/ml where 37.54±0.55 and 66.13±0.12 was found for the CGLE and CGFE respectively (Table 4).

Table 1: Phytochemical screening of ethanolic extract of *Couroupita guianensis*

Phytoconstituents	CGLE	CGFÉ
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Proteins	+	+
Saponins	-	-
Terpenoids	-	-
phenolics	+	+

(+) Presence of constituents; (-) Absence of constituents

***In vitro* antioxidant activity**

Table 6: Reducing power ability

Group	Concentration (µg/ml)	Absorbance at 700 nm
CGLE	50	0.131 ± 0.008
	100	0.233 ± 0.004
	200	0.289 ± 0.002
	400	0.379 ± 0.007
	800	0.487 ± 0.003
CGFE	50	0.068± 0.007
	100	0.130 ± 0.003
	200	0.176 ± 0.004
	400	0.230 ± 0.003
	800	0.313 ± 0.004
BHT (Standard)	50	0.241 ± 0.003
	100	0.323 ± 0.005
	200	0.497 ± 0.009
	400	0.971 ± 0.006
	800	1.531 ± 0.003

Values are mean ± S.E.M. (n=3), P<0.01 when compared with control

Table 7: Superoxide radical scavenging (NBT reduction) assay

Group	Concentration (µg/ml)	Absorbance at 560 nm	% inhibition	IC $_{50}$ µg/ml
Control		0.2117±0.0001		
CGLE	25	0.186 ± 0.0002	17.57 ± 3.222	188.39 ± 0.42
	50	0.153 ± 0.0004	25.55 ± 0.191	
	100	0.136 ± 0.0005	35.48 ± 0.246	
	200	0.098 ± 0.0004	53.54 ± 0.220	
	400	0.061 ± 0.0004	70.88 ± 0.212	
CGFÉ	25	0.189 ± 0.0002	10.46 ± 0.111	318.13 ± 0.21
	50	0.172 ± 0.0002	18.75 ± 0.126	
	100	0.148 ± 0.0001	29.81 ± 0.082	
	200	0.128 ± 0.0003	39.21 ± 0.147	
	400	0.092 ± 0.0003	56.41 ± 0.150	
Ascorbic acid (standard)	25	0.099 ± 0.004	33.33 ± 0.035	92.46 ± 0.54
	50	0.054 ± 0.006	63.87 ± 0.458	
	100	0.034 ± 0.003	76.78 ± 0.214	
	200	0.021 ± 0.002	85.79 ± 0.177	
	400	0.012 ± 0.002	91.82 ± 0.143	

Values are mean ± S.E.M. (n=3), p<0.01 when compared with control.

Table.8 Hydroxyl scavenging (Deoxyribose degradation) assay

Group	Concentration (µg/ml)	Absorbance at 532 nm	% inhibition	IC $_{50}$ µg/ml
Control		0.5732±.010		
CGLE	5	0.501 ± 0.0002	12.56 ± 0.040	37.54±0.55
	10	0.424 ± 0.0001	25.88 ± 0.020	
	20	0.354 ± 0.0001	38.22 ± 0.028	
	40	0.272 ± 0.0002	52.47± 0.040	
	80	0.203 ± 0.0002	64.52 ± 0.049	
CGFÉ	5	0.511 ± 0.0095	12.26 ± 0.141	66.13±0.12
	10	0.462 ± 0.0001	19.34 ± 0.028	
	20	0.394 ± 0.0002	31.19 ± 0.051	
	40	0.328 ± 0.0001	42.75 ± 0.031	
	80	0.247 ± 0.0002	56.76 ± 0.040	
Quercetin (standard)	5	0.646 ± 0.003	19.79 ± 0.393	24.5±0.29
	10	0.522± 0.001	35.16 ± 0.216	
	20	0.340 ± 0.003	57.81 ± 0.288	
	40	0.211 ± 0.004	73.75 ± 0.231	
	80	0.062 ± 0.001	92.26 ± 0.218	

Values are mean ± S.E.M. (n=3), p<0.01 when compared with control.

Conclusion

The hypothesis of obtaining plant based medicine is beneficial to human health based on the active profile exposed through various *in vitro* assays it can be concluded that the ethanolic extract of leaves and flowers of *Couroupita guianensis* showed significant antioxidant activities. Further investigations on the isolation and identification of Bio active components on the plant would help to ascertain its potency.

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