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Research Article

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Invitro antioxidant activity of methanolic extract of flower of *Couroupita guinensis* Aubl

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ABSTRACT

Antioxidant activity of methanolic extract of Couroupita guianensis flower was studied for its free radical scavenging property on different invitro models e.g.-1, 1-diphenyl-2-picrylhydrazyl (DPPH) method, hydrogen peroxide method and total antioxidant method. All the antioxidant activities were compared with standard antioxidant ascorbic acid. The values of the methanolic extract of Couroupita guianensis flowers were found to be 67.3±0.4µg in DPPH assay, 62.5±1.2µg in hydrogen peroxide model and 55.9±1.9µg in total antioxidant activity model at the concentration of 200µg/ml. It was concluded that couroupita guinensis flower possessed a strong antioxidant activity in the method of DPPH radical scavenging activity.

Keywords: Antioxidant activity, *Couroupita guianensis*, methanolic extract, DPPH assay, Hydrogen peroxide, Total antioxidant activity**.**

INTRODUCTION

Plants are the basis of life on earth and are central to people's livelihoods [1]. In recent times, there is an increasing interest in the role of free radical- mediated damage in the etiology of human diseases. In normal metabolism, the levels of oxidants (i.e. free radicals) and antioxidants in humans are maintained in balance, for sustaining optimal physiological conditions [2]. Overproduction of free radicals in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins [3] and thus leads to a range of chronic diseases, such as cardiovascular disease, neuronal disease, cataracts, and several forms of cancer [4]. It is established that the intake of antioxidant substances reinforces defenses against free radicals. The use of synthetic antioxidants has been limited because of their toxicity [5]. Therefore, it is of great significant and necessity that research focuses on discovering potential natural, effective antioxidants to replace the synthetic ones.

*Couroupita guinensis*is widely cultivated for its large showy flowers and reddish - brown woody capsular fruits upto 20 cm in diameter. It is grown in Indian gardens as an ornamental tree. It is native to south India and Malaysia and is commonly known as Nagalinga pushpam in tamil. The flowers, which are borne only on special stems on the main trunk, are orange, scarlet or pink, forming racemes up to 3m long [6]. 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[7,8].Based on the above-mentioned traditional uses of *Couroupita guinensis*, the present study was undertaken to evaluate the *invitro* antioxidant activity.

EXPERIMENTAL SECTION

Identification and collection of flower

The flowers of *Couroupita guinensis* were collected from the Mannargudi, Thiruvarur District, Tamilnadu, India. They were identified and authenticated by Dr. John Britto, The Rapient Herbarium and Centre for Molecular Systematics, St. Joseph's college, Trichirapalli, Tamilnadu, India.

Extraction and preparation of flower

The flowers were garbled and dried under shade and powdered. 25g of dried powdered flower materials were extracted separately with methanol using soxhlet apparatus for 48hrs.The solvent was distilled at lower temperature under reduced pressure and concentrated on water bath to get the crude extract which is stored in desiccator for future use.

invitro antioxidant activity

DPPH radical scavenging activity

The ability of the plant extract to scavenge 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radicals was assessed by the standard method[9].The stock solution of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 50, 100, 150, 200 µg/ml. Diluted solutions (1 ml each) were mixed with 3 ml of methanolic solution of DPPH (DPPH, 0.004%). After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured by reading the absorbance at 517nm using UV-Visible Spectrophotometer. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as control. Ascorbic acid was used as standard. The experiment was carried out in triplicate. The data were presented as mean values ± standard deviation (n = 3)

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \quad \text{equation (1)}$$

Hydrogen peroxide scavenging activity

Scavenging activity of Hydrogen peroxide (H_2O_2) by the plant extract was determined by the

Hydrogen peroxide scavenging activity

Scavenging activity of Hydrogen peroxide (H_2O_2) by the plant extract was determined by the method[10].Plant extract (4 ml) prepared in distilled water at various concentration(50, 100, 150, 200 µg/ml) was mixed with 0.6 ml of 4 mM H_2O_2solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using Eq. (1).

$$\%\text{inhibition} = \left(1 - \frac{(\text{Absorbance of the presence of the Sample of extract and standard})}{\text{Absorbance of the control}}\right) \times 100$$

Total antioxidant activity

For total antioxidant activity assay[11]various concentrations of the substrate dissolved in water were combined in an eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the methanol solution was measured at 695 nm against a blank.

A = (c ×V) / m
A =Total content of antioxidant compounds, mg/g plant extract, in ascorbic acid equivalent,
c= The concentration of ascorbic acid established from the calibration curve, mg/ml,
V = The volume of extract (ml), and
M = The Weight of crude plant extract (g).

RESULTS AND DISCUSSION

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissue and biomolecules, eventually leading to disease conditions, especially degenerative diseases. Many plant extracts and phytochemicals have been shown to have antioxidant / free radical scavenging properties. Antioxidant activity of methanolic extract of *Couroupita guinensis* flower were determined by three different *invitro* methods.

1. DPPH (1, 1-Diphenyl, 2-picryl-hydrazyl) assay
2. Hydrogen peroxide scavenging activity
3. Total antioxidant activity

These methods are most popular *invitro* assays for determination of antioxidant activity.

TABLE: 1 *invitro* antioxidant activity of *Couroupita guianensis* flower

S.no	Concentration in µg/ml	Ascorbic acid (standard)	DPPH assay	Hydrogen peroxide activity	Total antioxidant activity
1	**50**	**20.1±0.1**	**30.1±2.6**	**27.3±0.8**	**22.6±0.5**
2	**100**	**34.0±1.2**	**43.4±2.1**	**41.9±2.2**	**34.1±3.2**
3	**150**	**45.5±1.3**	**54.1±0.9**	**53.4±0.9**	**46.7±1.6**
4	**200**	**50.8±1.7**	**67.3±0.4**	**62.5±1.2**	**55.9±1.9**

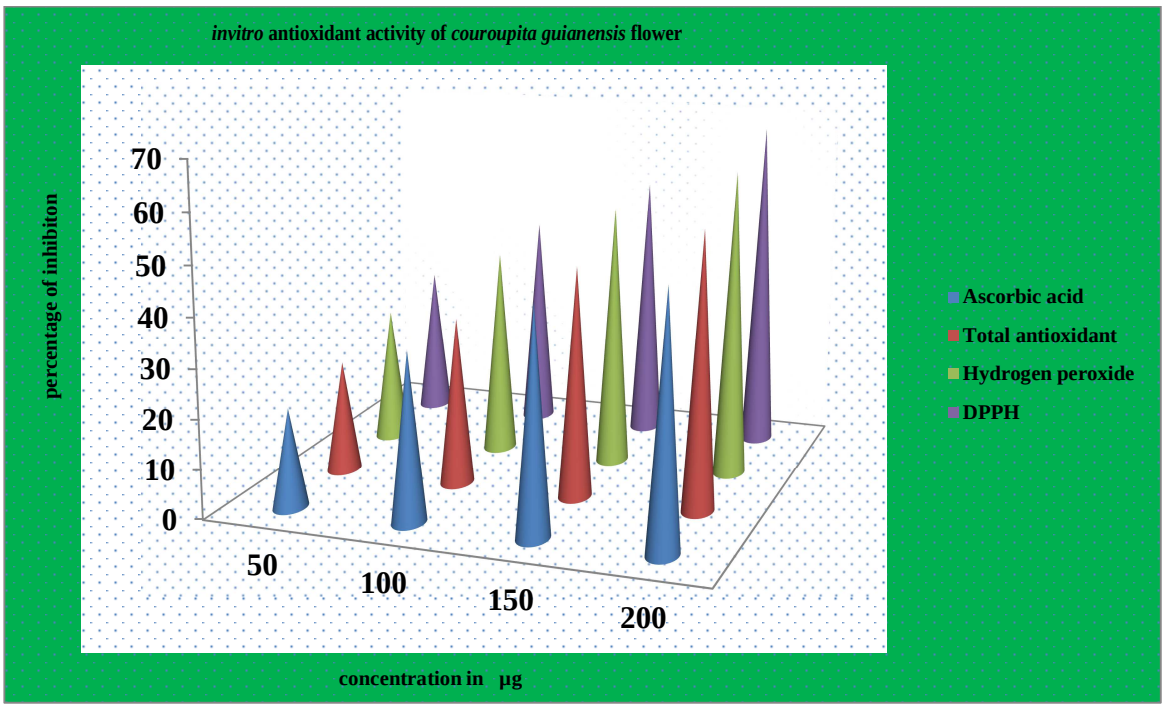

Fig: 1 Graphical analysis of *invitro* method

DPPH assay

Couroupita guianensis exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentration tested (50,100,150 and 200µg/ml) there was a dose dependent increase in the percentage antioxidant activity for all concentrations tested. The extract at a concentration of 50µg/ml showed a percentage inhibition of 30.1±2.6 and for 200µg/ml it was 67.3±0.4.Ascorbic acid at a concentration of 50µg/ml exhibited a percentage inhibition of 20.1±0.1 and for 200µg/ml 50.8±1.7 (Table 1).A graded increase in percentage of inhibition was observed for the increase in the concentration of ascorbic acid.

The methanolic extract of C*oleous vettiveroides* exhibited a maximum DPPH scavenging activity of 55.08% at 1000 Fg/ml whereas for Rutin (standard) it was found to be 61.75% at 1000 Fg/ml. The IC_{50} of the methanol extract of C*oleous vettiveroides* and Rutin were found to be 250 µg/ml and 800µg/ml respectively[12].

Ethyl acetate extraction of water extract of C*ouroupita guianensis* flower showed a concentration dependent antiradical activity by inhibiting DPPH radical with an EC_{50} value of 24.41µg/ml.Ethyl acetate fraction of water extract of C*ouroupita guianensis* showed almost two times more inhibitory activity on DPPH radical than the standard curcumin which showed an EC_{50} value of52.71 µg/ml[13].

The aqueous extract of *Nyctanthes arbor-tristis*(L) flower of showed maximum activity of 33.6±0.6 and 37.6±0.62 respectively at 150 and 200 µg/ml were as ascorbic acid at the same concentration exhibited 22.3±0.3 and 26.6±2.3 inhibition respectively. This result indicated that extract has a noticeable effect on scavenging the free radical by the method of DPPH assay[14].

DPPH is long –lived nitrogen radical. Antioxidants react quickly with DPPH and tend to decrease its oxidation ability. The natural antioxidants might directly react with or quench the stable cation radical, which is reflected as their antioxidant activity. The excellent reducing power of the sample may be due to the hydrogen donating abilities of the active constituents. More antioxidant activity in herbs comes from the ingredients other potentially important antioxidants. The phenolic compounds are dominant antioxidants attributed widely in the plant kingdom that exhibit scavenging efficiency on free radicals[15].

DPPH is a purple colored stable free radical; when reduced it becomes the yellow-colored diphenyl-picryl hydrazine. DPPH radicals react with suitable reducing agents and then electrons become paired-off and the solution loses colour stoichimetrically with the number of electrons taken up[16].

DPPH radical scavenging method is standard procedure applied to the evaluation of antiradical activity. The DPPH free radicals, which are stable in ethanol shows maximum a proton donating substances such as antioxidant, the radicals would be scavenged and absorbed [17].

In free radical scavenging activity, DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm, which is induced by different antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation [18].

The DPPH assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidative potential of various natural products [19].

Hydrogen peroxide activity

The percentage of hydrogen peroxide activity of methanolic extract of C*ouroupita guianensis* is presented in Table 1. The methanolic extract of C*ouroupita guianensis* exhibited a maximum activity of 62.5±1.2at 200 µg/ml whereas for ascorbic acid (standard) it was found to be 50.8±1.2 at 200 µg/ml.

The percentage of H_2O_2 scavenging activity of aqueous extract of *Nyctanthes arbor-tristis*(L) was found to be 27.3±0.8 which is highest among the concentration of 200µg/ml compared to antioxidant activity of standard ascorbic acid[14].

The *Couroupita guinensis* extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. At the concentration of 1 mg/mL, the DPPH scavenging activity for aqueous extract, ethanolic extract and ascorbic acid was 63.70, 67.47 and 80.59%, respectively. The IC_{50} values for aqueous extract, ethanolic extract and ascorbic acid were 97.78, 82.04 and 3.22 respectively[20].

Hydrogen peroxide a biologically reagent, non- radical oxidizing species, may be formed in tissues through oxidative processes. Hydrogen peroxide which in turn generate hydroxyl radicals (OH) resulting in initiation and propagation of lipid peroxidation. The Hydrogen peroxide scavenging activity of n-hexane extract of C*itrullus lanatus*, cholorofrom extract of C*itrullus lanatus* and ethanol extract of C*itrullus lanatus* seeds were detected and compared with ascorbic acid. The IC_{50} values for hydrogen peroxide scavenging activity of for hexane extract maximum followed by ethanol extract and for chloroform extract was minimum. Though the extracts showed good hydrogen peroxide scavenging activity but it was less effective than standard ascorbic acid. The ability of the extracts to quench OH-seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction [21].

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally – occurring iron complexes inside the cell believed to react with H_2O_2*invivo* to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects [22].

Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems [23].Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells[24].

Total antioxidant activity

Total antioxidant activity is based on the scavenging ability of the extract as well as ascorbic acid, which is used as standard. As shown in fig. 1 the total antioxidant activity was found to increase in dose dependent manner. At this maximum concentration (200µg/ml), inhibition for methanolic extract of C*ouroupita guianensis* and ascorbic acid was found to be 55.9+1.9 and 50.9±1.7.

Total antioxidant activity of aqueous extract of *Nyctanthes arbor -tristis*(L)flower was performed at different concentration ranging from 50 -200 µg/ml.The percentage of H_2O_2 scavenging activity of aqueous extract was found to be 34.1±3.2 which is highest among the concentration of 200µg/ml compared to antioxidant activity of standard ascorbic acid[14].

Total antioxidant activity of the methanolic extract of *Coleous vettiveroides* was determined by phosphomolybdate method. The free radical scavenging potential shown maximum activity is 72% at 1000Fg/ml; for as Standard (ascorbate) it was found to be 69% at 1000 Fg/ml. The $IC_{50}$of the methanolice xtract of *coleous vettiveroides* and standard (ascorbate) was found to be 560Fg/ml and 585Fg/ml better antioxidant respectively [12].

CONCLUSION

Global search is going on a vast scale to identify pharmacologically potent antioxidant compounds with low profile of side effects for food and health industry. Numerous sources like plants, animals and synthetic chemical preparations can be utilized for derivation of antioxidants. But safety and ethical issues are reason for concern associated with synthetic and animal – derived antioxidants, respectively.

The results of the above investigation indicated that the methanolic extract of C*ouroupita guianensis* flower showed strong antioxidant activity. Further work on isolation and identification of active compounds and its efficacy needs to be done.

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