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ANTIOXIDANT STATUS OF LEAVES OF *CAESALPINIA BONDUC*

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ABSTRACT:

In modern era due to the life style adopted by man the free radical production in human body is rather increasing. The fast food that comprises junk and deep fried items along with the lack of physical activity is posing danger to human health and life. The free radical production is effectively combated by the use of antioxidants. The present study was aimed to determine the antioxidant activity of ethanol and methanol leaf extracts of wild thorny shrub *Caesalpinia bonduc* (L) Roxb., belonging to the family Caesalpinaceae. As free radicals exist in different forms the antioxidant activities of leaves were measured by five different assays with suitable control and standard. The assays include DPPH (free radical scavenging), FRAP (to asses reducing power of iron), superoxide radical scavenging (O_2^-), nitric oxide scavenging and hydrogen per oxide scavenging. Ethanol leaf extract had exhibited 83.733±0.123% of inhibition for DPPH assay which was measured spectrophotometrically . The corresponding IC_{50} values were also calculated. The results of four other assays when compared with standard have given better results thereby proving the antioxidant potential of leaves of the plant. These studies had brought much insight in pharmacognostic drug development from plant. Since the antioxidant activity of plant has proved to be evident the cytotoxicity assays can be carried out in future.

Keywords: *Caesalpinia bonduc,* free radicals, antioxidant activity, DPPH assay

[I] INTRODUCTION

Caesalpinia bonduc (L) Roxb., is a wild highly thorny shrub belonging to the family Caesalpinaceae. It is commonly called as the Gray Nicker Bean. It is a free-flowering and free-fruiting plant without periodicity [1]. The plant can thrive well on sea shores [2]. The traditional medical practices brought to light the ethanomedical uses of young leaves of *Caesalpinia bonducella* Flemming (Caesalpiniaceae) to treat certain tumors in few remote villages of Kolli Hills in Nammakal District of Tamil Nadu in India [3]. A number of chemical constituents flavonoids, phenols, diterepenoids and steroids have been reported from leaves, seeds and roots of the plant [4-8]. These compounds may exert multiple biological activities including antioxidant activity.

The free radicals are produced as the by - product of cellular metabolism in the cells or due to environmental effects. They exist as independent molecules or atoms that contain one or more unpaired electrons that are highly reactive. It is estimated about 1-4% of the oxygen taken up by the body is converted to reactive oxygen species. They include free radicals in the form of either superoxide (O_2^-) or hydroxyl radical (OH^-) or peroxyl radicals (ROO^-) and non free radicals in the form of hydrogen per oxide (H_2O_2) [9,10]. All these molecules are highly reactive and react with all major biomolecules such as proteins, lipids, nucleic acids, etc.

$$O_2 + e^- \rightarrow O_2^{\cdot -}$$

The above way specifies the endogenous method of the free radical production while the exogenous sources of the free radicals include tobacco smoke, ionizing radiation, pollutants, organic solvents and pesticides [11-13]. The oxidative stress produced by free radicals can be overcome by the inbuilt antioxidant system available in the body or by the use of antioxidants.

The natural antioxidant mechanism may be inefficient and hence the dietary intake of antioxidant compounds becomes important [14]. Currently, there is a growing interest toward natural antioxidants of herbal resources [15-17]. Owing to the complexity of the antioxidant materials and their mechanism of actions, it is obvious that no single testing method is capable of providing a comprehensive picture of the antioxidant profile of samples and a combination of different methods is necessary[14].

[II] MATERIALS AND METHODS

2.1. Collection and Processing of plant samples

The healthy leaves of *C. bonduc* were collected from Thandarai Village, Kanchipuram district, Tamil Nadu. The collected samples were air dried and powdered into a uniform powder. The extracts of samples were prepared by soaking 100 g of dried powder in 1:1 ratio of ethanol and methanol separately for 12 h by cold extraction method. The extracts were filtered using Whatman filter paper no. 42. The crude extract was concentrated in vacuum at 40^0 C in rotatory evaporator. The extract was diluted to get 1mg/ml of the sample and used for assays.

2.2. Antioxidant assays

2.2.1. DPPH assay

The antioxidant activity or free radical scavenging activity of ethanol and methanol leaf extract of *Caesalpinia bonduc* against DPPH (1,1-Diphenyl-2,Picryl-Hydrazyl) was measured [18]. DPPH is a stable free radical that accepts an electron or hydrogen radical there by reducing itself to become a stable diamagnetic molecule [19]. The DPPH is often used as a substrate to evaluate anti oxidative activity of antioxidants [20]. DPPH with purple color is reduced to yellow colored diphenyl picryl hydrazine when the antioxidants are present in the sample which is measured at 517 nm. The positive control and the negative controls were also prepared. The readings were taken at different time intervals of 5-30 mins along with standard and control.

The percentage inhibition of DPPH radical by the sample was calculated using the following formula

Inhibition % = $(A_c - A_s) / A_c \times 100$

A_c - Absorbance of control

A_s - Absorbance of sample

Ascorbic acid was taken as the standard.

2.2.2. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried for different fractions of ethanol extract of *Caesalpinia bonduc* [21]. 100µl of sample was added to 3 ml of the FRAP reagent. The mixture was homogenized and the absorbance of reaction mixture was then detected at 593 nm after 4 min in room temperature. The standard curve was constructed using ferrous sulphate solution, and the results were expressed as µmol Fe (II)/g dry weight of plant material.

2.2.3. Superoxide Radical Scavenging Assay

The superoxide radical scavenging activity of *C.bonduc* was measured by standard method using NBT solution [22]. The absorbance at 560nm was measured against quercitin as control and ascorbic acid as standard. All the tests were performed in triplets. The percentage of inhibition was calculated as per the formula of DPPH assay.

2.2.4. Nitric oxide scavenging assay

The nitric oxide radical inhibition was estimated by the use of Griess Illosvoy reaction [23] for ethanol extract of *C.bonduc* with positive control and standard. The negative control was also

prepared with distilled water and the reagent. The absorbance was measured at 540 nm. Then the percentage of inhibition was calculated.

2.2.5. Scavenging of Hydrogen Peroxide (H_2O_2) radicals

The scavenging of hydrogen peroxide radical was measured for various fractions of ethanol leaf extract of *Caesalpinia bonduc* **[**24,25]. The solution of hydrogen peroxide was prepared in PBS (pH 7.4). The sample (1mg/ml) was added to hydrogen peroxide. The absorbance at 230 nm was determined 10min later against a blank and percentage inhibition was calculated.

2.2.6 Statistical Analysis

All tests were performed in triplets and the results were expressed in mean± standard deviation, where n=3.

[III] RESULTS AND DISCUSSION There is an inverse relationship between the dietary intake of antioxidant rich food and the incidence of the human disease [26]. The ethanol and the methanol leaf extracts of *Caesalpinia bonduc* had shown the gradual increase in dose dependant manner for the DPPH activity in terms of percentage inhibition depicted in fig 1. The ethanol extract had shown percentage of inhibition ranging from 55.48± 3.72% 83.733±0.123 % at 30 minutes period of incubation. The methanol extract for DPPH test had given the result ranging from 44.05%-57.01%.

The IC_{50} values obtained indicates that ethanol extract of the plant could serve as an effective antioxidant. The IC_{50} values of ethanol extract of seeds of *Caesalpinia bonduc* was 74.73µg/ml [27] while in present study IC_{50} of ethanol leaf extract of C*aesalpinia bonduc* was 50µg/ml and for methanol extract IC_{50} was 150µg/ml. Therefore ethanol extract of leaves were taken up for further antioxidant studies. The IC_{50} values of leaves had concluded *C.bonduc* was supposed to possess a strong antioxidant activity even more than the seeds.

The results of all other antioxidant assays have been listed in table 1. The FRAP assay was determined for ethanol extract of *Caesalpinia bonduc* based on DPPH results. The ferric reducing antioxidant power for ethanol extract of *Caesalpinia bonduc* had shown that there is an effective role of extract in the reducing antioxidant power of iron. The result of the FRAP in (µmol Fe(II)/g have given the value as 0.52 for the extract while 0.58 for the standard used.

The nitric oxide scavenging activity of ethanol extracts of *Caesalpinia bonduc* the percentage inhibition of 58.16±0.37 when compared to the standard ascorbic acid to be as 36.71±3.21 for the concentration of 25µg/ml. The seed extracts of *Caesalpinia bonduc* have given the result as 102.65 g/ml [27]. The IC_{50} value of nitric oxide scavenging activity of the methanol leaf extract of C*aesalpinia bonducella* Flem., was 102.8g/ml when compared with the standard curcumin 20.4g/ml [3].

The estimation of antioxidant activity of ethanol extract of *Caesalpinia bonduc* by the method of the superoxide scavenging radical assay had shown that the sample had 41.89±0.33 while the activity of the standard was 55.47±1.55% of inhibition.

The scavenging of hydrogen peroxide radicals for the ethanol extract *Caesalpinia bonduc* was 51.17±0.14%. The ethanol extract of seeds of *Caesalpinia bonducella* also inhibited the hydroxyl radical and superoxide anions with IC_{50} values of 109.85 and 89.84l g/ml respectively [27]. All the methods mentioned in table 1 have shown that 50% of activity was reached even at 25 µg/ml of extract that proves the leaves are evident in curing the degenerative diseases than the other parts of the same plant.

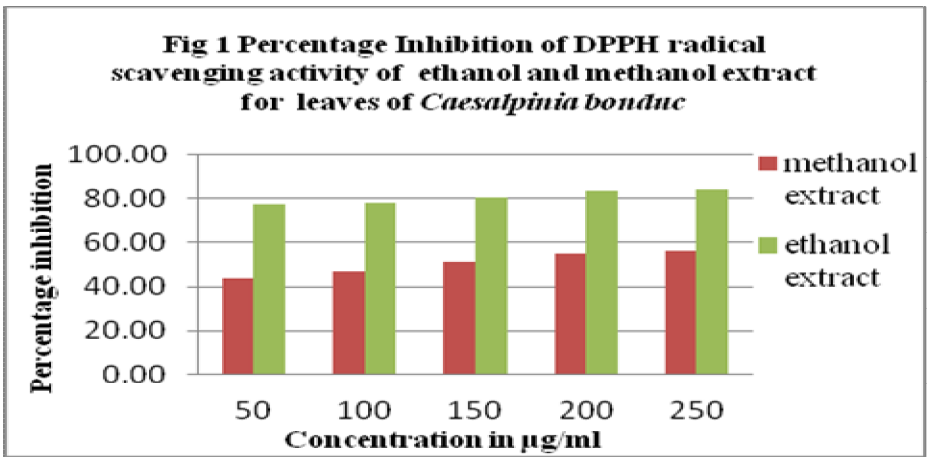

Fig1: DPPH activity in terms of percentage inhibition depicted.

S.No	Concentration (µg/ml)	Super oxide radical scavenging		Nitric oxide scavenging		Scavenging of hydrogen peroxide	
		Ascorbic acid	Ethanol extract	Ascorbic acid	Ethanol extract	Ascorbic acid	Ethanol extract
1	5	10.44±0.11	7.02±0.36	13.81±3.78	21.71±0.04	34.27±3.92	13.00±4.73
2	10	17.35±0.87	14.54±0.40	19.01±0.399	26.88±0.45	41.34±0.94	31.60±1.18
3	15	30.49±1.43	22.25±0.93	24.42±0.374	44.88±0.20	46.93±2.79	42.55±1.38
4	20	41.22±1.68	30.60±0.70	33.04±0.312	48.78±0.26	51.05±0.26	47.02±1.79
5	25	55.47±1.55	41.90±0.33	36.71±0.320	58.17±0.38	55.17±0.93	51.17±0.14

Table 1 Free radical scavenging activity of ethanol extract of *Caesalpinia bonduc*

Values represent mean ± SD, n=3

[V] CONCLUSION

The potential antioxidant activity was confirmed by DPPH for ethanol leaf extract of *Caesalpinia bonduc.* All other antioxidant activities studied substantiate the promising antioxidant capacity of leaf extract to completely remove all the available different forms of the free radicals. The data provided had enriched the existing data of antioxidant activity of the plant. The results also suggest that the antioxidant activity may contribute to the cytotoxic nature of the plant which may be the future study to be carried out.

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