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Research Article

PHYTOCHEMICAL SCREENING, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *CEIBA PENTANDRA*

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ABSTRACT

The seed of *Ceiba pentandra* collected from the rural area of Shirur, Pune district, were dried, milled and extracted with ether, ethanol and water. Phytochemical screening was carried out according to standard procedures. Carbohydrate & flavonoids, fixed oil, glycosides were found to be present in the aqueous, ether & alcoholic extract resp. Total Flavonoid content was determined by spectrophotometric methods and that was found to be 2.56 g & 2.20g quercetin equivalent per 100 g of ethanolic & water extract resp. *In-vitro* antioxidant activity of ethanolic seed extract of *Ceiba pentandra* was determined by DPPH free radical scavanging assay. All the analysis was made with the use of UV-Visible Spectrophotometer (Jasco V-530). The ethanolic seed extracts of *Ceiba pentandra* had shown very significant DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. In DPPH free radical scavanging assay IC_{50} value of ethanolic seed extracts of *Ceiba pentandra* was found to be 50.33±5.29 as compared with standard ascorbic acid (44.93±2.92 µg/ml).

Keywords: Ceiba pentandra, DPPH, free radical scavenging, total flavonoid.

INTRODUCTION

Reactive free radicals, including superoxide, hydroxyl radical, and peroxyl radical, generally result in degradation of protein, lipid peroxidation, and oxidation of DNA, which have been considered to be linked with many chronic diseases, such as diabetes, cancer, and atherosclerosis[1]. Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties[2, 3]. *Ceiba pentandra* is an evergreen tree belonged to Malvaceae family. Originally a native to South America it now has spread to the primary rainforests of West Africa, and the Southeast Asian rainforests of the Malay Peninsula, and the Indonesian archipelago. In many places the straight trunks of the kapok tree are used to make dugout canoes. The white, fluffy seed covering is used in pillows and mattresses. Since it is buoyant and water resistant it is often used in flotation devices and padding. The seeds, leaves, bark and resin have been used to treat dysentery, fever, asthma, and kidney disease. In Mayan myths the kapok tree was sacred[4,5]. The aim of this experiment is to investigate free radical scavenging activity of the Seed aqueous and alcohol extracts of *Ceiba pentandra* using DPPH method, and to quantitate total flavonoid content using a spectrophotometric method.

MATERIALS AND METHOD

Plant Material: Samples of the seeds of *Ceiba pentandra* were collected from the rural area of Shirur, Pune district, in the month of sept-oct 2010. The samples were identified by Mr. R.P.Ganorkar, Head of Botanical Department of C.T.Bora College, Shirur (Ghodnadi). Dist-Pune, Maharashtra. Samples were cleaned and air dried in then powdered and passed through a sieve with mesh number 20.

Chemicals and Instruments: Quercetin, DPPH and ascorbic acid were obtained from Hi Media Labs, Mumbai. Aluminium chloride was purchased from Research lab Mumbai. All organic solvents were of analytical grade and supplied from Research Lab, Mumbai. UV-Visible Spectrophotometer (JascoV-530) was used for antioxidant activity determination by DPPH method and for total flavonoid content determination.

Preparation of extract: Dried powder of seed samples were extracted by using Soxhlet Extractor petroleum ether and ethanol as a solvent. Also the water extract obtained by maceration method.

Total Flavonoid Content Determination

5 ml of 2% aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of *Ceiba Pentandra* seed extracts (0.02 mg/ml). Absorption readings at 415 nm were taken after 10 minutes against a blank sample without $AlCl_3$. The total flavonoid content was determined using a standard curve of quercetin (0.01-0.1 mg/ml). The mean of three readings was used and expressed as mg quercetin equivalent (QE)/100 g extract[6].

Antioxidant activity

Free radical scavenging activity of compounds was determined using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Briefly, 2 mL extract and standard of various concentrations (10-100µg/mL) were added to 2 mL of 100 µM DPPH solution[7]. After 20 minute incubation at room temperature, the absorbance was read against a blank at 517nm. The change in absorbance with respect to the control (containing DPPH only without sample, expressed as 100% free radicals) was calculated as percentage scavenging using following the equation:

$$(A517_{blank} - A517_{sample}) \div A517_{blank} \times 100\%.$$

The reading was taken in triplicate and mean used for calculation of IC_{50}. The IC_{50} (mean ± SEM) stand for the concentration required for 50% inhibition of DPPH radicals and was calculated from ORIGIN PC version 6.0 software.

Assay of reducing power

This was carried out as per the method of Yildrim *et al*. and Lu and Foo.1[12,13] ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer(0.2M, pH 6.6) and 2.5 ml potassium ferricyanide $[K_3Fe(CN)_6]$ (10g/l), then mixture was incubated at 50^0C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which

was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml FeCl3 (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer (Jasco V-530 UV-Visible Spectrophotometer 117, INDIA). Ascorbic acid was used as standard and phosphate buffer used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power[8].

Table 1: Preliminary Phytochemical Screening of *Ceiba Pentandra*

Test	Ethanol Extract	Aqueous Extract	Petroleum ether extract
Alkaloids	-	-	-
Carbohydrate	-	+	-
Proteins	-	-	-
Glycoside	+	-	-
Flavonoids	-	+	-
Tannins and Phenolic compounds	+	+	-
Fixed oil	-	-	+

Table 2: Absorbance of Sample For Total Flavonoid Content

Sample	Concentration (mg/ml)	Absorbance
Standard	0.01	0.1060
Standard	0.02	0.2172
Standard	0.04	0.4306
Standard	0.06	0.7028
Standard	0.08	0.7906
Standard	0.1	0.9912
Test (alc. Extract)	1	0.2828
Test (water Extract)	1	0.2300

Table 3: % Radical Scavenging Activity of Alcoholic Extract

Sr. No.	Conc. µg/ml	Standard (Ascorbic acid)		Test (Alcoholic extract)	
		Absorbance±SEM	% RSA±SEM	Absorbance±SEM	% RSA±SEM
1	10	0.878±0.017	36.53±1.06	0.958±0.057	30.79±3.98
2	20	0.833±0.017	39.78±1.11	0.872±0.030	36.98±2.03
3	30	0.760±0.019	45.06±1.26	0.804±0.046	41.87±3.18
4	40	0.704±0.023	49.11±1.57	0.737±0.042	46.74±2.91
5	50	0.658±0.020	52.47±1.36	0.678±0.027	50.98±1.84
6	60	0.614±0.018	55.64±1.18	0.631±0.30	54.41±2.10
7	70	0.570±0.011	58.80±0.73	0.572±0.036	58.63±2.50
8	80	0.511±0.009	63.08±0.56	0.527±0.022	61.91±1.48
9	90	0.467±0.015	66.24±1.04	0.482±0.015	65.17±0.99
10	100	0.408±0.014	70.52±0.94	0.425±0.01	69.27±1.24
11	Blank	1.384±0.003		1.384±0.003	

Table 4: Antioxidant Activity Expressed In IC_{50}

Sample	DPPH scavenging activity IC_{50}(± SEM) (µg/ml)[a]
Test (Alcoholic extract)	50.33±5.29
Standard (Ascorbic acid)	44.93±2.92

[a] The results are expressed as IC_{50}±SEM (n=3) (µg), the concentration of the test compound that provides 50% scavenging of the DPPH radicals already available in the solution.

Table 5: Absorbance for assay of Reducing Power

S.No	Concentration (µg/ml)	Absorbance of standard	Absorbance of test
1	100	0.1251	0.0894
2	200	0.1664	0.1129
3	300	0.1953	0.1485
4	400	0.2102	0.1622
5	500	0.2420	0.1763

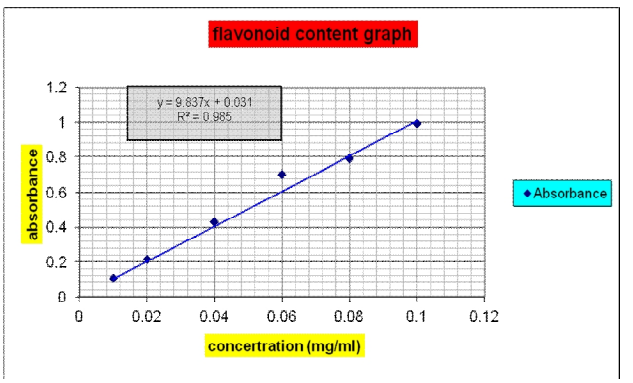

Figure I: Graph of absorbance against concentration for total flavonoid content

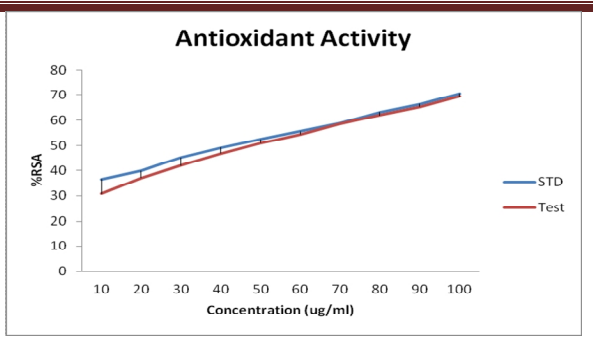

Figure 2: Graph of % scavenging of DPPH against Concentration

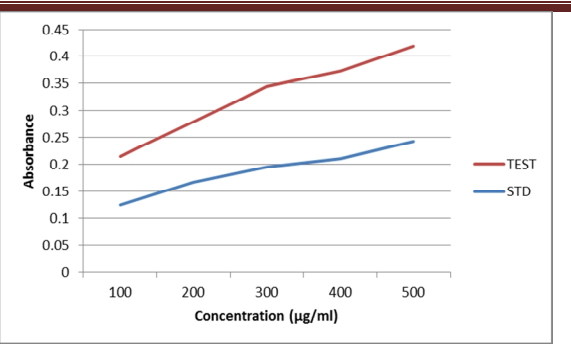

Figure 3: Graph for assay of reducing power

RESULT AND DISCUSSION

Phytochemical screening: The ether, alcohol and aqueous extracts of *Ceiba pentandra* were subjected to preliminary phytochemical studies[9,10]. It has been observed that ethanol, pet ether, and aqueous extracts contains chemical constituent like glycoside, fixed oils, carbohydrate and flavonoids respectively (Table I).

Total Flavonoid content: Total Flavonoid content was determined by spectrophotometric method. The obtained observations are mentioned in table 2 and plotting graph absorbance vs concentration (in figure 1). Total flavonoid content was found to be 2.56 g & 2.20g quercetin equivalent per 100 g ethanolic & water extract resp. By using the equation (y = 9.837x + 0.031).

Antioxidant activity:

In order to determine the extent of scavenging effect, ethanol extract of the seed of *Ceiba pentandra* was tested for antioxidant activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Extract was showed antioxidant activity (Table 3 and 4). Extract showed 50.33±5.29 IC_{50} as compared with standard ascorbic acid (44.93±2.92 µg).

Assay of reducing power

The reductive capabilities of the *Ceiba pentandra* leaves extract was compared to ascorbic acid. The reducing power of *Ceiba pentandra* leaves extracts was very potent and the power of the extract was increased with quantity of sample (Table 5 & Figure 3).

CONCLUSION

This study confirms that seed of *Ceiba pentandra* tree contains high amount of flavonoid content and the plant bears good antioxidant activity.

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