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Estimation Of Phytoconstituents (Total Phenol And Total Flavonoid Content) And In Vitro Antioxidant Activity Of *Ceiba Pentandra* Linn. Bark

Khyati Shah[1*], ResaParmar[2], Dhruvanshi Gol[3], Bhanubhai Suhagia[4]

[1*]Department of Pharmacognosy, Government Pharmacy College, Gandhinagar, 382028, Gujarat, India.
[2]Department of Pharmacognosy, B. K. Mody Government Pharmacy College, Rajkot, 360003, Gujarat, India.
[3]Department of Pharmaceutical Quality Assurance, B. K. Mody Government Pharmacy college, Rajkot, 360003, Rajkot, India.
[4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, 387001, Gujarat, India.

***Corresponding author:**Dr.Khyati N. Shah
*Department of Pharmacognosy, Government Pharmacy College Gandhinagar, Polytechnic campus, Sector 26, Tata Chokdi, Gandhinagar: 382028, Gujarat, India. Email id: Khyati_ns@yahoo.co.in
Mobile no: 8511115937

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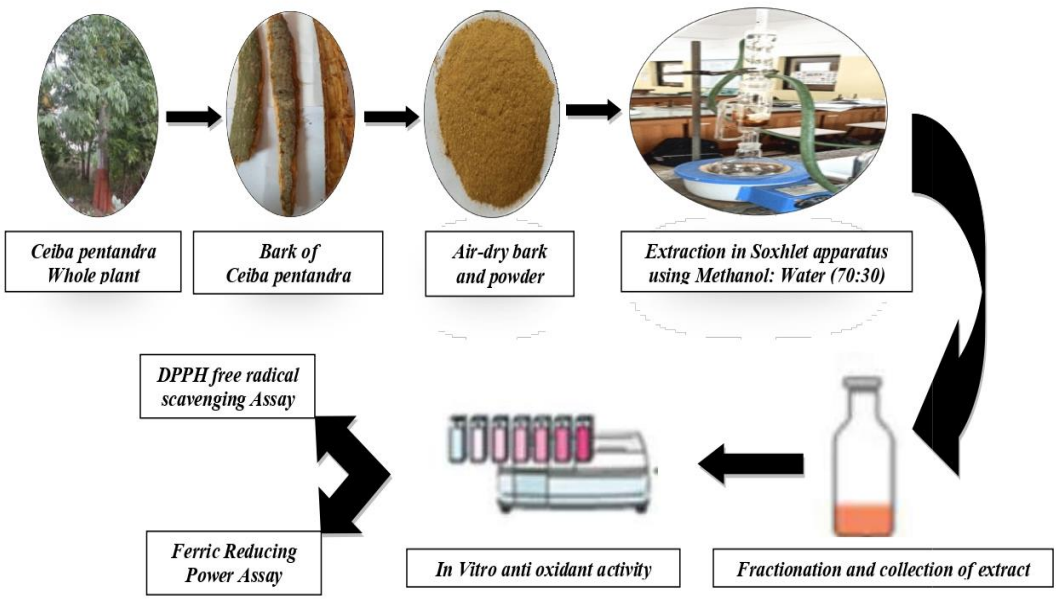

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Abstract

Because of their capacity to lower reactive oxygen species formation and hence prevent various age related illnesses, characterization of antioxidant-active chemical is of significant interest. Antioxidants have the capacity to protect organisms from oxidative stress generated by free radicals. A lot of study is being done throughout the world to uncover natural antioxidants in various herbal origin medications. The reported study include phyto-chemical screening, determination of poly-phenolic content and antioxidant activity using different in vitro models of the hydro-methanolic fraction of *Ceiba pentandra* Linn. bark. The anti-oxidant activity was scrutinized spectrophotometrically. Phyto-chemical screening revealed the presence of Phenolics, Tannins, Saponins, Flavonoids, Steroids and Carbohydrates. The total phenolic content and total flavonoid content was found to be 239.11 ± 0.769 mg GAE/gm dry extract and 201.66 ± 1.154 mg RTE/gm dry extract respectively. In DPPH scavenging activity percentage inhibition was found to be in the range of 25.64 to 73.69 for extract of *Ceiba pentandra* Linn. with IC_{50} value 141.74 µg/ml and in Ferric Reducing Power Assay extract showed dose dependent increase in absorbance indicating good antioxidant activity. Our findings show that hydro-methanolic extract of *Ceiba pentandra* Linn. is a possible source of natural antioxidants, which validates its use in folkloric medicine.

Keywords: *Ceiba pentandra* Linn., Total Phenolic Content, Total flavonoid content, DPPH free radical scavenging Assay, Ferric Reducing Power Assay

Introduction

The dangers of oxidative stress on human health have become a major concern. According to the World Health Organization (WHO), traditional medicine is used by 80 percent of the world's population for primary health care, and the majority of this therapy involves the use of plant extracts and their active compounds (Winston, 1999). Under stress, the human body produces more reactive oxygen species (ROS),such as superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide as well as enzymatic and non-enzymatic antioxidant (Arouma et al., 1987 ; Lefer and Granger, 2000). Endogenous antioxidants are antioxidants that are generated inside the system of living organisms and repair free radical damage internally by beginning cell regeneration. Exogenous antioxidents, on the other hand, are acquired from sources outside of living systems, such as food supplements, and they externally induce cell repair (Jaouad and Torsten, 2010; Wolfe et al., 2003).

This imbalance causes cell damage and health issues. The development of cardiovascular illnesses, malignancies(Gerber et al., 2002),neurological illness (Matteo and Esposito, 2003)and inflammatory illnesses (Sreejayan and Rao, 1996)such as degenerative disease is aided by a shortage of antioxidants, which can quench reactive free radicals. Supplementing the diet with antioxidant chemicals found in natural sources is one answer to this problem. As a result, these natural antioxidant sources can act as a source of preventive medicine. Exogenous antioxidant supplementation has become more common as the necessity to balance these endogenous antioxidants has grown. Exogenous antioxidant from natural sources are currently attracting a lot of attention and study, presumably because they are less costly, more easily available, and thought to have less negative effects than their synthetic equivalents (Tadhani et al., 2007).

*Ceiba pentandra*Linn.belongs to the family, Bombacaceae. The tree is a native of Malaya, occursin the forests throughout hotter parts of India (mainly western and southern India and the Andaman Islands) and Ceylon. It is distributed to South America, tropical Africa and in the West Indies. The stem bark have been reported to have anti-urolithiatic (Choubey et al., 2010), hypoglycemic (Satyaprakash et al., 2013), anti-ulcer (Anosike et al., 2013), anti-bacterial and anti-fungal (Asare and Adebayo, 2012; Nwachukwu et al., 2008), anti-diarrhoeal (Sule et al., 2009), anti- microbial

(Njinga et al., 2015)and hepatoprotective (Bairwa et al., 2010) amongst other medicinal properties. In addition, the presence of tannins, saponins, flavonoids, alkaloids, phenols, steroids, resin and carbohydratehave been reported in thedifferent extract of bark of *Ceiba pentandra* Linn (Anosike and Ofoegbu, 2013; Ezigbo et al., 2013).As per the traditional usesthe different parts of *Ceiba pentandra* Linn.are used in the management of various diseases like of liver, spleen and blood, and removes tumors, vertigo, rheumatism, diuretic and astringent(Kirtikar and Basu, 1975; Nadkarni, 1995; Chatterjee and Prakashi, 1997; Anonymous, 1992; Chopra et al., 1956; Chunekar and Pandey, 2013).The aim of this study was to evaluate the in vitro antioxidant activity of hydro-methanolic extract of bark of *Ceiba pentandra* Linn.

Materials and Methods

Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl),Folin-Ciocalteu reagent, Ascorbic acid and Gallic acid standards were purchased from (Sigma- Aldrich). The Solvents used for extraction were analytical grade (Sigma Aldrich) and other chemicals used for various tests were of analytical grade (Sigma Aldrich).

Collection and Identification of plant material

The bark of *Ceibapentandra* Linn.was collected from mature branches of the tree from University road, Rajkot, India in the month of July and authentication was confirmed by a taxonomist at Department of Biosciences, Saurashtra University, Rajkot, India. The dried bark sample was powdered and stored in the air tight container at room temperature for further use.

Preliminary phytochemical screening

Successive solvent extraction was done for preliminary phytochemical screening using increasing polarity solvents i.e., Petroleum ether (60-80°C), Toluene, Chloroform, Ethyl acetate and Methanol. The material was dried each time, before changing the solvent. At end, the marc was macerated with the chloroform water for 24 hours to obtain the aqueous extract. After removal of solvents using rotary evaporator, the extracts were dried on water bath. The extracts obtained from successive solvent extraction were screened for detection of presence or absence of various phytoconstituents (Kokate, 2014).

Extraction of *Ceiba pentandra* bark powder for total phenol content and antioxidant activity

Based on presence of maximum phytoconstituents in methanol and water extracts obtained in successive solvent extraction and presence of phenolics and flavonoids in above extracts, hydroalcoholic extract was prepared for estimation purpose. Fine bark powder of *Ceiba pentandra* Linn. (50 gm) was extracted exhaustively with 70% hydromethanolic solvent in a Soxhlet apparatus. The solvent was completely removed by rotary evaporator and weight of residue as well as percentage yield was recorded. Dried extract was stored in airtight container under cool condition. This crude extract was used for further investigation for potential antioxidant properties.

Estimation of Phytoconstituents

Estimation of total phenolics

The total Phenolic content of the dry extracts of *Ceibapentandra* Linn.was determined by Folin-Ciocalteu method (Giri et al., 2013). Standard and sample readings were measure by using a spectrophotometer at 765nm against the blank.For preparation of test sample accurately weighed

10 mg of dried hydro methanolic extract was dissolved in 10 ml methanol:water (70:30) to give 1 mg/ml stock solution, from which 3 ml solution was taken and farther diluted up to 10 ml and this diluted solution was used as a test sample. From which the test sample of*Ceibapentandra* Linn. (0.5ml) was mixed with 5 ml of Folin-Ciocalteu's (FC) phenol reagent (1:10) and 4 ml of Na_2CO_3 solution (7.5 %w/v in water) was added and vortex for 5 minutes. The reaction mixture was kept in the dark place for 30min at 40℃ and; the absorbance was measured at 765nm in a spectrophotometer. Total phenolic content was calculated as mg GAE/gm dry extract on the basis of a standard calibration curve of gallic acid (20, 40, 60, 80 and 100 μg/ml) plotted using by same procedureand amount of total phenolics was expressed in mg GAE/ gm dry extract. Each assay was performed in triplicates.

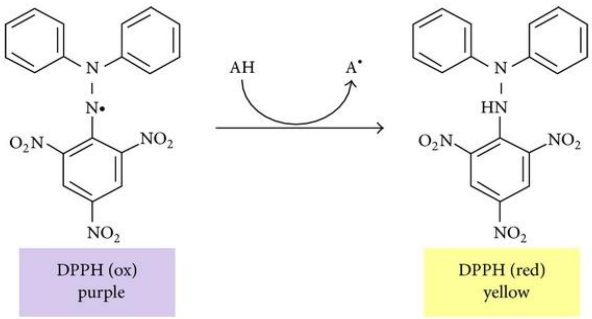
Estimation of total flavonoids

Accurately weighed 0.1 gm dried hydroalcoholic extract of was dissolved in 100 ml methanol: water (70:30) to give 1mg/ml solution. The total flavonoid content was determined using aluminum chloride method. 1 ml of the extract was mixed with 4 ml distilled water and to which 0.30 ml of 10% NaNO2 solution was added. After 5 minutes 0.3 ml 10% AlCl3 was added followed by addition of 2 ml 1M NaOH solution. Total volume was made up to 10 ml with distilled water. The absorbance was measured at 510 nm using colorimeter against blank (prepared without addition of AlCl3). Total flavonoids were calculated from the calibration curve of Rutin (20, 40, 60, 80 and 100 μg/ml) by following same procedure and amount of total flavonoids was expressed in mg RTE/ gm dry extract (Kavitha and Indira, 2016; Saeed et al., 2012). The estimation was carried out in triplicate.

Antioxidant Potential

DPPH free radical scavenging Assay

Principle of the scavenging reaction between (DPPH°) and an antioxidant (H-A) can be written as: Antioxidants react with DPPH° which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance decreased from the DPPH° radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. Chemical reaction is shown below.



DPPH assay method was used for determining the antioxidant activity. DPPH (α, α-diphenyl-β-picrylhydrazyl, $C_{18}H_{12}N_5O_6$, M=394.33) is a stable free radical, which get reduced by hydrogen atom from antioxidants and convert to corresponding hydrazine.

The % Scavenging effect of various concentrations of hydromethanolic extract of *Ceibapentandra* Linn.was measured by using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay (Yadav et al., 2018).The 3 ml of freshly prepared 0.1mMDPPH solution and2 ml of sample (ascorbic acid and

extract of *Ceibapentandra* Linn. in the range of 25-400 µg/ml) was mixed. These solutions wereincubated for 30 min in a dark and cool place. The resulting solution was shaken dynamically. The absorbance was measured at 517 nm using a spectrophotometer against blank solution.The percentage inhibition of DPPH radical scavenging property of the sample was calculated using the following equation:

$$\text{DPPH radical scavenging activity} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100\%.$$

Reducing power by $FeCl_3$

Ferric reducing capacity of extract was determined using potassium ferricyanide-ferric chloride method (Wang et al., 2009). 1 ml of sample (ascorbic acid and extract of *Ceibapentandra* Linn.in therange of 20-100 µg/ml) was mixedwith 2.5 ml of 0.2 Mphosphate buffer (6.6 pH) and 2.5 ml of 1% potassium ferricyanide.This solution was kept at 50°C for 20 min.After incubation2.5 ml of aqueous 10% trichloroaceticacid was added and was centrifuged at 3000 rpm for 10 minutes. From this 5 ml solution was mixed with 5 ml of distilled water. To this 1 ml of 0.1% Ferric Chloride was added and it was incubated 35°C for 20 min. Absorbance was measured at 700 nm against blank (prepared omitting extract and $FeCl_3$) and directly used to express the reducing power. Higherabsorbance of the reaction mixture indicates greater reducing power. Each extract was assayed in the triplicate for each concentration.

Result

Collection and Identification of plant material

The collected plant material was dried and powdered as mentioned in materials and methods. Figure 1 shows whole plant, collected and dried bark and powdered bark.

Figure 1: whole plant, collected and dried bark and powdered bark

Preliminary Phytochemical Screening

The phyto-chemical analysis is of paramount importance in identifying a new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically

investigated. Preliminary phyto-chemical screening of the extract showed the presence of primary and secondary metabolites like carbohydrates, steroids, phenolics, saponins and flavonoids. Thus from these biochemical investigations, it is quite evident that *Ceibapentandra* Linn.is very rich source of secondary metabolites. The result of the phyto-chemical test has been summarized in Table 1.

Table 1: Preliminary phytochemical screening of the *Ceiba pentandra*bark powder((+) indicate presence and (-) indicate absence)

Chemical constituent	Petroleum ether	Toluene	Chloroform	Ethyl acetate	Methanol	Water
Alkaloids	-	-	-	-	-	-
Phenolics	-	-	-	+	+	+
Flavonoids	-	-	-	+	+	-
Tannins	-	-	-	-	+	+
Condensed Tannins	-	-	-	-	+	+
Saponin	-	-	-	-	+	+
Sterols and triterpenoids	+	+	+	+	-	-
Coumarins	-	-	+	+	-	-
Carbohydrates	-	-	-	-	+	+
Proteins	-	-	-	-	+	+
Anthraquinone glycosides	-	-	-	-	-	-

Extract yield

Total of 2.59gms of hydroalcoholic dried extract was obtained from 50 grams of powdered drug with 5.18 % w/w yield.

Estimation of phytoconsituents

Estimation of total phenolics

Gallic acid was used as reference standard and the calibration curve of the same (in concentration range of 20, 40, 60, 80 and 100 μg/ml) was found to be linear with correlation coefficient (R2 = 0.9961) and linear regression equation was Y= 0.039x + 0.1268. The total phenolic content was determined using mentioned calibration curve, and was found to be 239.11 ± 0.769 mg GAE/gm dry extract.

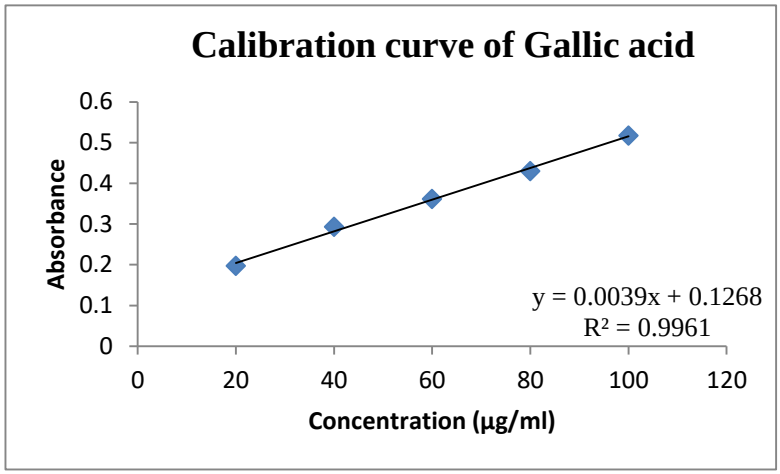


Figure 2: Calibration curve of standard Gallic acid

Estimation of total flavonoids

Rutin was used as reference standard and the calibration curve of the same was found to be linear with correlation coefficient (R2 = 0.9966) and linear regression equation was Y = 0.0017x + 0.018. The total flavonoids content was determined using above calibration curve, and was found to be 201.66 ± 1.154 mg RTE/gm dry extract.

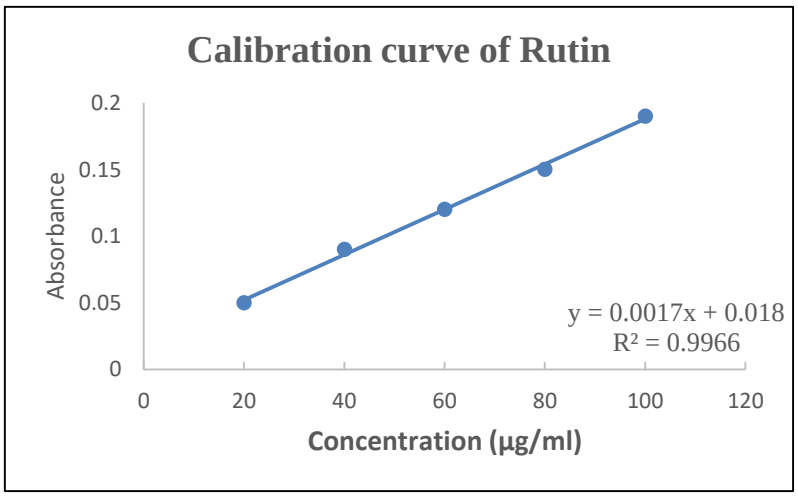


Figure 3: Calibration curve of standard Rutin

Table 2: Estimation of phytoconstituents

Phytoconstituent	Amount± SD
Total Phenolics (mg GAE/gm dry extract)	239.11 ± 0.769
Total Flavonoids (mg RTE/gm dry extract)	201.66 ± 1.154

Anti-oxidant Activity

Plants rich in secondary metabolites including phenolics, flavonoids and carotenoids show evidence of antioxidant activities which are due to their redox properties and chemical structures. The antioxidant property of the crude extracts was investigated by various biochemical assays like, DPPH and FRAP.

DPPH free radical scavenging activity

The hydro-methanolic extract of bark demonstrated antioxidant activity. InDPPH scavenging activity percentage inhibition was found to be in the range of 25.64 to 73.69 for extract of Ceibapentandra Linn. as well as in the range 43.69 to 88.33 for ascorbic acid, for concentration range of 25-400 µg/ml.IC_{50} value was obtained 141.74 µg/ml and 61.34µg/ml for hydro methanolic bark extract of *Ceiba pentandra* Linn. andascorbic acid respectively. Percentage inhibition and relationship between concentrations vs. percentage inhibitions is shown in figure 2.

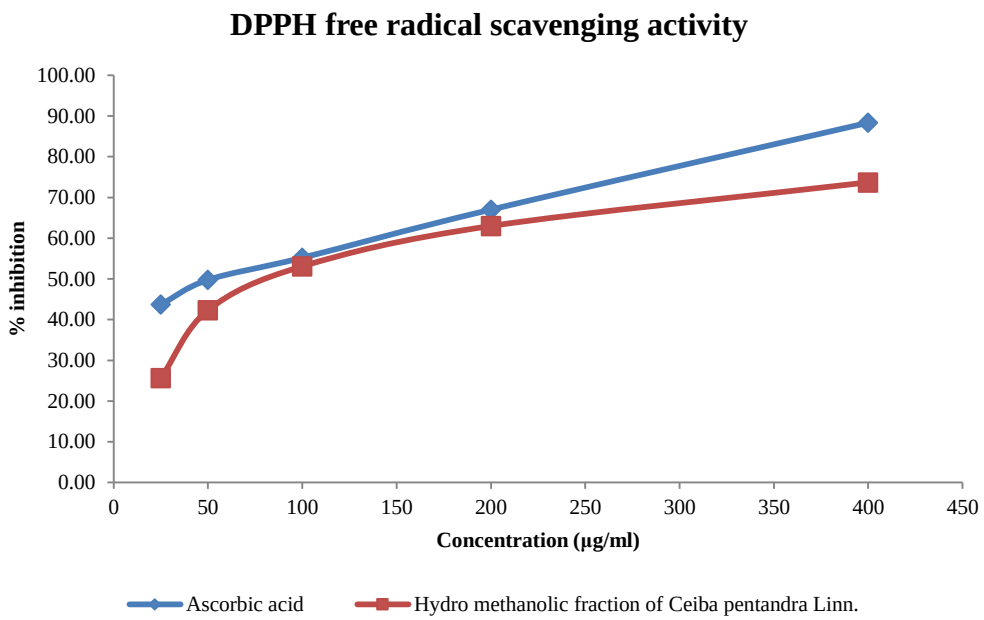


Figure 4: Relationship betweenpercentage inhibitionfor standard and *Ceiba pentandra* Linn.extract vs. concentration

Reducing power by $FeCl_3$

Presence of antioxidant substances or reductants in the bark extracts leads to the reduction of Fe^{3+} ferricyanide complex to the ferrous form (Fe^{2+}). The reducing power of the crude extract of *Ceibapentandra* Linn.was also evaluated and significant increase in the absorbance was observed with increase in concentration of the sample (ascorbic acid and extract of *Ceiba pentandra* Linn. in the range of 20-100 µg/ml). For bark extract absorbance values rangedfrom 0.18 to 0.56. Ascorbic acid was used as a positive control. Results of Reducing power by $FeCl_3$ shows good antioxidant property of extract. Data for reducing power by $FeCl_3$ and relationship between concentrations vs. absorbance is shown in figure 3.

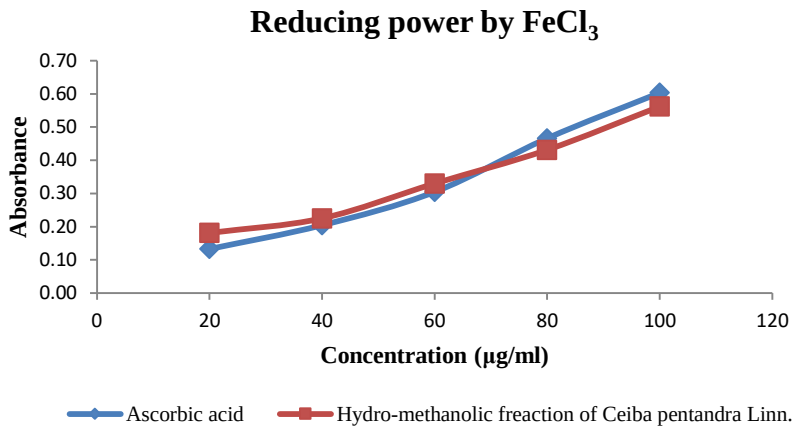


Figure 5: Relationship between absorbance for standard and *Ceiba pentandra* Linn.extract vs. concentration

Discussion

Phyto-chemical studies portray the presence of several biologically active secondary metabolites such as Phenolics, Tannins, Saponins, Flavonoids, Steroids and Carbohydratesin the bark of*Ceibapentandra* Linn. In our study we determined the total phenolic and anti-oxidant potential of hydro-alcoholic extract of bark of *Ceibapentandra* Linn.wherethe extract shows high phenolic content and high flavonoid content as well as good antioxidant capacity. The significant phenolic and flavonoid content of the crude extract may explain its antioxidant properties. Because of their potential to scavenge free radicals assisted by their hydroxyl groups, phenolic compounds are key plant elements and the total phenolic might be utilized as a foundation for quick screening of antioxidant activity (Baba and Malik, 2014). Flavonoids, on the other hand, inhibit the generation of reactive oxygen, chelate trace elements involved in free radical generation, scavenge reactive species, and up regulate and preserve antioxidant defenses (Bravo, 1998; Agati et al., 2012). Crude extract of polyphenolics and flavonoids rich fruits, herbs, vegetables, cereals, and other plant materials are increasingly being employed in the food sector for their antioxidant qualities and health advantage.

Conclusion

Because of the consequences for human health replacing synthetic antioxidants with natural antioxidants may be advantageous. Based on the present evaluation of antioxidant activity and total phenolic content of the bark of *Ceibapentandra*Linn, plant can serve as the potent source of natural antioxidants. The results of in vitro studies suggest that hydro-alcoholic extract of *Ceibapentandra* Linn.may be useful in defense against many diseases due to its excellent antioxidant properties.

Conflicts of Interest: Authors have no Conflicts of Interest.

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