

Evaluation of Phytochemicals and Antioxidant Activities of *Ceiba pentandra* (Kapok) Seed Oil

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Abstract

Background: *Ceiba pentandra* seeds belonging to order Malvales and the family Malvaceae, commonly known Kapok were evaluated for their phytochemical ingredients and antioxidant activity.

Materials and methods: Soxhlet extraction method was used for the extraction of oil, phytochemicals and antioxidant activities of *Ceiba pentandra* seed oil was estimated by well-known methods.

Results: The seed oil was extracted using soxhlet extraction method with analytical grade hexane as refluxing solvent. The oil was thick yellowish in colour having pungent odour with a yield of 40%. Phytochemical constituents and non enzymatic antioxidants showed enhancement in increasing concentration from 25 mg/mL to 100 mg/mL. At 100 mg/mL recorded phytochemical components phenols, flavonoids, alkaloids and tannins were 305 µg gallic acid equivalents g⁻¹, 3207 µg quercetin equivalents g⁻¹, 0.34 µg boldine equivalents g⁻¹ and 2041 µg tannic acid equivalents g⁻¹ respectively. The seed oil exhibited striking Diphenyl picryl hydrazyl radical scavenging Assay (DPPH), Ferric reducing or Antioxidant power assay (FRAP), reducing power assay and Hydroxyl radical scavenging activity. The measured DPPH activity at 100 mg/mL was 47.56% of inhibition per 50 µL of oil as compared to 45 and 76% of inhibition per 1 mg/mL of Rutin and BHT as positive controls. Observed FRAP ability of *Ceiba pentandra* seed oil was 309 FRAP units at 100 mg/mL concentration, reducing activity of *Ceiba pentandra* seed oil was observed at 100 mg/mL as 20.52 µg of ascorbic acid equivalents per mL of oil. Hydroxyl radical scavenging activity of *Ceiba pentandra* seed oil observed as 39.69% inhibition/0.1 of oil at 100 mg/mL, as compared to positive controls, Ascorbic acid and BHT at 1 mg/mL with percentage of inhibition 75 and 75.6 respectively.

Conclusion: The importance of the phytochemical constituents and non-enzymatic activities of *Ceiba pentandra* seed oil in the maintenance of health is strengthened as trend of the future is moving towards using seed oil as medicine in the management of various chronic diseases.

Keywords: DPPH; FRAP; Reducing power assay; Hydroxyl radical scavenging activity

Introduction

Free radicals are generated in our body during the normal metabolic processes and during exposure to adverse pathophysiological conditions [1]. They are unstable species that are able to induce cellular damage in several ways. The most deleterious effects of free radicals is damage to DNA [2], which is associated with the process of carcinogenesis. Phytochemicals such as phenolics, carotenoids and dietary fibers are gaining increased attention because of their antioxidant, anticarcinogenic, antimutagenic, and other health promoting properties [3]. Although synthetic antioxidants such as butylated hydroxy toluene, butylated hydroxy anisole, gallic acid esters and tertiary butylated hydroquinone have potential to neutralize free radicals, they have been criticized due to possible toxic effects, low solubility along with moderate antioxidant activity. Hence there arises a need to discover new potential natural sources of antioxidants.

Several studies have successfully correlated the phenolic content with antioxidant activity. For example, natural phytochemicals present in berry crops, tea, oilseeds, beans, fruits and vegetables, herbs and spices such as rosemary, thyme, sage, nutmeg, turmeric, white pepper, chili pepper, ginger and several medicinal plants are good source of antioxidant activity [4] and this antioxidant activity have been correlated to the plant secondary metabolites such as flavonoids, carotenoids, alkaloids, tannins and phenolic compounds.

Many fruits have inedible seeds and are not part of human diet.

However, such seeds are a part of ayurvedic preparations against many diseases. Further, since seeds are the primary stage of plant life cycle, they have strong defence mechanism possibly due to the presence of phytoconstituents contributing to antioxidant activity [5]. Seeds are the primary stage of plant life cycle, they have strong defence mechanism possibly due to the presence of phytoconstituents contributing to antioxidant activity. In the present study, the oil of *Ceiba pentandra* seeds (belonging to order Malvales and the family Malvaceae; commonly known Kapok) was evaluated for their phytochemical constituents and antioxidant activity.

Materials and Methods

Chemicals

Chemicals and reagents used for antioxidant estimations were purchased from Merck. All additional chemicals used were analytical grade. Altogether the experiments were performed at room temperature unless otherwise stated.

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Collection of seeds

Mature dried fruits of *Ceiba pentandra* were obtained in between April and June of 2011, from in and around Andhra University area, Visakhapatnam, India. Healthy seeds were selected, oil was extracted and used for the evaluation of phytochemical and antioxidant activities.

Extraction of oil by soxhalation

The seed oil was extracted using soxhlet extraction method with analytical grade Hexane as refluxing solvent. At the completion of the extraction process, the oil was recovered from the mixture by distillation and stored at 4°C until required use.

The percentage of oil content can be calculated as below:

$$\% \text{ of Oil} = \frac{\text{Wt. of Oil obtained in gm}}{\text{Wt. of Seed taken in gm}} \times 100$$

Evaluation of phytochemicals

Estimation of total phenolics: The amount of total phenolics in extracts was determined according to the Folin- ciocalteu procedure [6]. Samples (200 μ L) were introduced into test tubes. One milliliter of Folin ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) in micrograms per gram of extract as calculated from standard gallic acid graph.

Estimation of total flavonoids: Total flavonoid content of the extract was determined according to a modified colorimetric method [7]. Seed extract (1.0 mL) was mixed with 1 mL of distilled water and 75 μ L of a 5% NaNO_2 solution. After 5 min, 75 μ L of 10% $\text{AlCl}_3 \cdot \text{H}_2\text{O}$ solution was added. After 5 min, 0.5 mL of 1M Sodium hydroxide was added. The solution was mixed well and kept for 15 min. The increase in absorbance was measured at 510 nm using a UV-Visible spectrophotometer. The total flavonoid content was calculated using standard quercetin calibration curve. The results were expressed as micrograms of quercetin equivalents (QE) per gram of extract.

Estimation of total tannins: The total tannins were determined using the Folin-ciocalteu method [8], briefly, 0.1 mL of seed extract, 6.5 mL of water and 0.5 mL of Folin-ciocalteu reagent and 1.5 mL of 20% sodium carbonate at overnight standard solution were added and incubated at 1 h. The absorbance of sample was measured in spectrophotometer at 725 nm. The total tannin content was calculated using standard tannic acid calibration curve and the results were expressed as micrograms of tannic acid equivalents per gram of extract.

Estimation of total alkaloids: Total alkaloid content was estimated by the method of Sreevidya and Mehrotra [9]. A standard solution was prepared by dissolving 5 mg of boldine and seed extract separately in 5 mL of warm distilled water each. 5 mL of boldine solution/extract was adjusted to pH 2-2.5 (with 0.01 M HCl), and 2 mL of DR (Dragendorff's reagent) was added to form an orange precipitate that was centrifuged at 5000 rpm for 15 min. Afterward, DR was added to the supernatant to check for complete precipitation. 2 mL amount of 1% sodium sulfide was added to the residue to form a brownish black precipitate which was centrifuged at 5000 rpm for 15 min. Complete precipitation was checked by further adding 1% sodium sulfide. The resulting residue was dissolved in 2 mL of nitric acid with warming and sonication and then made up to 10 mL with distilled water. 5 mL of 3% thiourea was added to 1 mL of the resulting solution to form a yellow bismuth complex, of which the absorbance was measured at 435 nm. All the

assays were performed in triplicate. The amount of bismuth present in the boldine solution/extract was achieved from the calibration curve of bismuth nitrate. The results were expressed as boldine, considering that is a monobasic alkaloid, and therefore the complex formed with bismuth follows a 1:1 stoichiometry.

Evaluation of Non-Enzymatic Antioxidants

Diphenyl picryl hydrazyl radical scavenging assay (DPPH)

3 ml of seed extract was added to 1ml of 0.1 mM solution of DPPH in methanol. After 30 min incubation at 37°C absorbance was measured at 517 nm against control using a spectrophotometer [10]. Rutin and BHT were used as the reference materials. The percentage of inhibition was calculated by comparing the absorbance values of the test samples with those of the controls.

The inhibition percentage (I) was calculated as radical scavenging activity as follows:

$$\text{Percentage of Inhibition (I)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

Ferric reducing or antioxidant power assay (FRAP)

The total antioxidant power of the sample was assayed by the method of Benzie and Strain [11]. 3.0 mL of FRAP working reagent was taken in a test tube then 100 μ L of plant extract was added, this is vortex mixed, and the absorbance was read at 593 nm against a reagent blank at a predetermined time after sample-reagent mixture. The results are expressed as ascorbic acid equivalents (μ moles/mL) or FRAP units.

Determination of iron (III) to iron (II) reducing activity (or) reducing power assay

The ability of the extracts to reduce iron (III) was assessed by the method of Oyaizu [12]. One mL of seed extract was mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6, and 2.5 mL of 1% aqueous potassium hexacyanoferrate [$\text{K}_3\text{Fe}(\text{CN})_6$] solution. After 30 min of incubation at 50°C, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of water and 0.5 mL of 0.1% aqueous FeCl_3 , and the absorbance was recorded at 700 nm using a UV-visible spectrophotometer. Increasing absorbance at 700 nm was interpreted as increasing reducing activity. The results were expressed as micrograms of ascorbic acid equivalents (AscAE) per gm of extract. Butylated hydroxy toluene (BHT) and ascorbic acid were used as positive controls. Ascorbic acid was used as the standard control with concentrations 10, 20, 40, 60, 80 and 100 μ g/mL.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+} /Ascorbate/EDTA/ H_2O_2 system, a method carried out by Gulhan et al. [13]. 0.1 mL of the seed extract was added to the reaction mixture containing 0.1 mL of 3.0 mM deoxyribose, 0.5 mL of 0.1 mM FeCl_3 , 0.5 mL of 0.1 mM EDTA, 0.5 mL of 0.1 mM ascorbic acid, 0.5 mL of 1 mM H_2O_2 and 0.8 mL of 20 mM phosphate buffer, pH 7.4, in a final volume of 3.0 mL. The reaction mixture was incubated at 37°C for 1 h. The formed thiobarbituric acid reactive substances (TBARS) were measured by adding 1.0 mL of thiobarbituric acid (TBA) and 1.0 mL of trichloroacetic acid (TCA) to the test tubes and incubated at 100°C for 20 min. After the mixtures were cooled, absorbance was measured at 532 nm against a control containing

deoxyribose and buffer. A blank was carried out similar way as the test except test compound.

Inhibition (I) of deoxyribose degradation in percent was calculated in the following way:

$$I = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

Statistical analysis

The results of *in vitro* study were given as Mean \pm Standard Deviation (SD) obtained from three independent experiments, and analyzed with Student's t-test for paired data and a 'p' value less than 0.05 was considered as significant difference in the analysis.

Results

Evaluation of phytochemicals

Oil extraction was carried out by soxhlet extraction method as per the direction of AOAC (Association of Official Analytical Chemists). Hexane was used as solvent for extracting the oil from seeds and it was passed out for 10 h. The oil was thick yellowish in colour having pungent odour with a yield of 40% (Figure 1).

Quantitative analysis of *Ceiba pentandra* seed oil was showed



Figure 1: *Ceiba pentandra* seed oil.

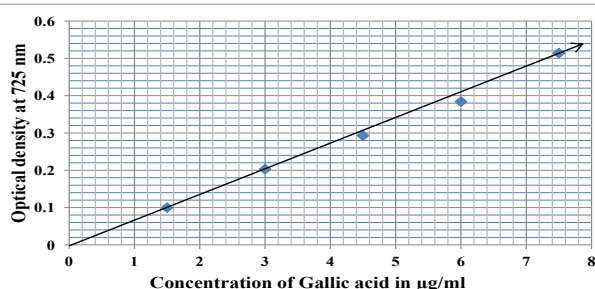


Figure 2: Gallic acid standard graph.

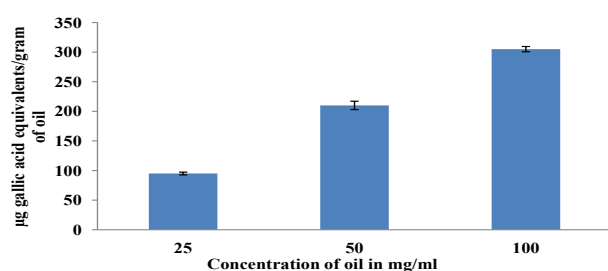


Figure 3: Phenolic content of *Ceiba pentandra* seed oil.

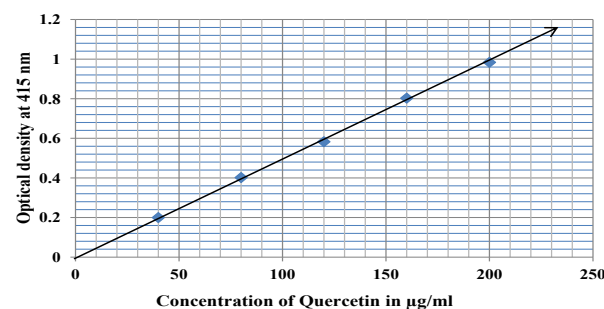


Figure 4: Quercetin standard graph.

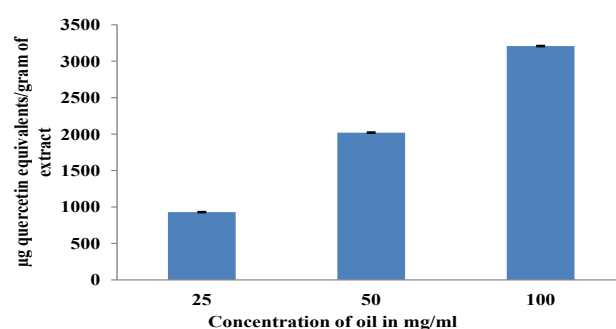


Figure 5: Flavonoid content of *Ceiba pentandra* seed oil.

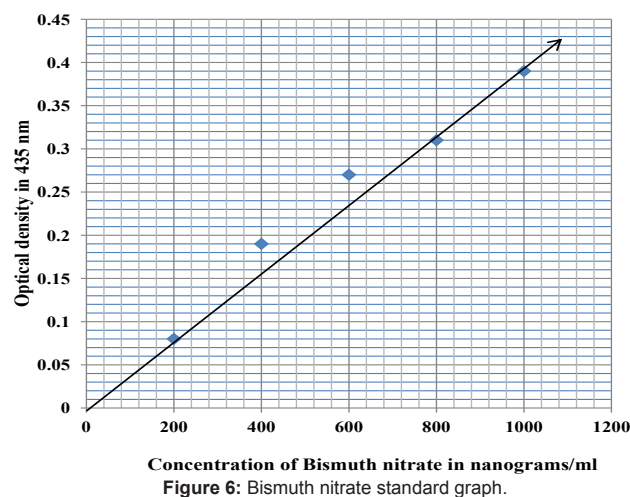


Figure 6: Bismuth nitrate standard graph.

enhancement in increasing concentrations from 25 mg/mL to 100 mg/mL. The observed phenolic content of *Ceiba pentandra* seed oil at 100 mg/mL was 305 µg gallic acid equivalents g⁻¹. The graphical versions were showed in Figures 2 and 3.

The screened flavonoid content of *Ceiba pentandra* seed oil at 100 mg/mL was 3207 µg quercetin equivalents g⁻¹. Graphical reports were put on view in Figures 4 and 5. Moreover, the screened alkaloid content observed at 100 mg/mL in the seed oil was 0.34 µg boldine equivalents g⁻¹, outcome was put on show in Figures 6 and 7.

The estimated tannin content of *Ceiba pentandra* seed oil was 2041 µg tannic acid equivalents g⁻¹ at 100 mg/mL. Graphical view was showed in Figures 8 and 9.

Evaluation of non-enzymatic antioxidants

All measured antioxidant capacities of the *ceiba pentandra* seed oil were increased in a dose dependent manner ranged from 25-100 mg/mL. The measured DPPH activity at 100 mg/mL was 47.56% of inhibition per 50 μ L of oil as compared to 45 and 76% of inhibition per 1 mg/mL of Rutin and BHT as positive controls. Graph was exhibited in Figure 10.

Observed FRAP ability of *Ceiba pentandra* seed oil was 309 FRAP units at 100 mg/mL concentration, graphical appearance was showed in Figures 11 and 12.

Reducing activity of *Ceiba pentandra* seed oil was observed at 100

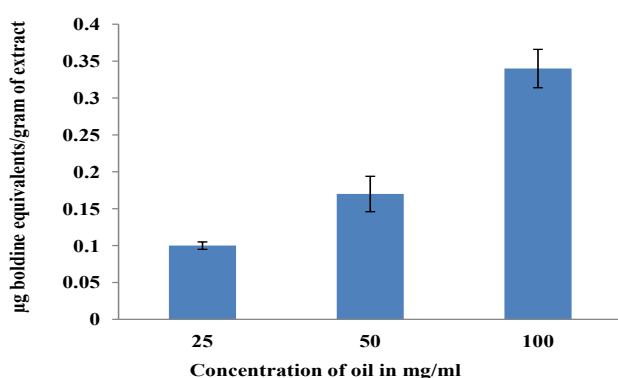


Figure 7: Alkaloid content of *Ceiba pentandra* seed oil.

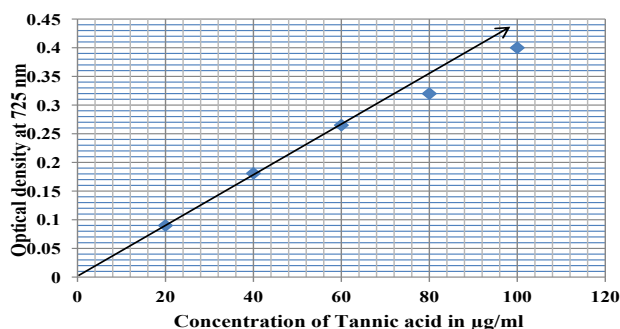


Figure 8: Tannic acid standard graph.

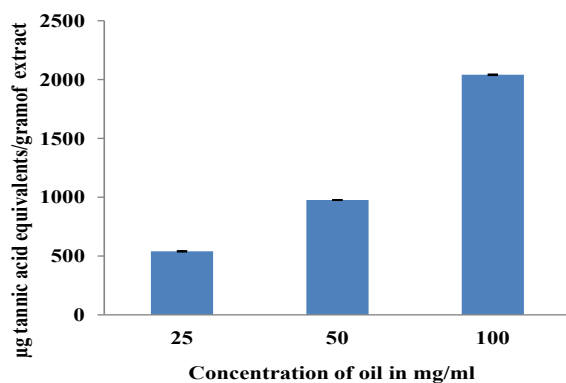


Figure 9: Tannin content of *Ceiba pentandra* seed oil.

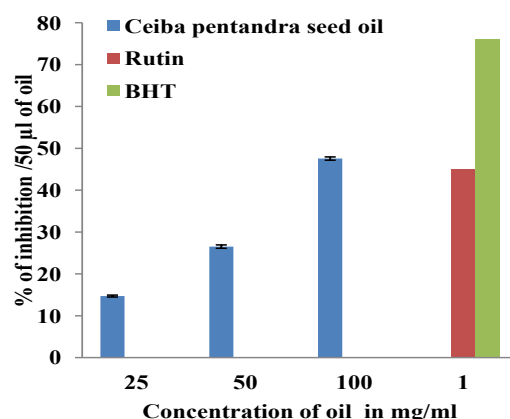


Figure 10: DPPH activity of *Ceiba pentandra* seed oil.

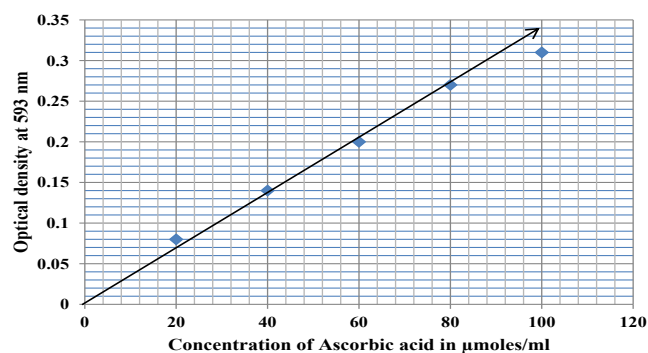


Figure 11: Ascorbic acid standard graph.

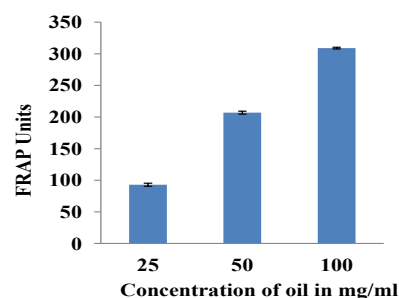


Figure 12: FRAP ability of *Ceiba pentandra* seed oil.

mg/mL as 20.52 μ g of ascorbic acid equivalents per mL of oil. Graphs were displayed in Figures 13 and 14.

Hydroxy radical scavenging activity of *Ceiba pentandra* seed oil observed as 39.69% inhibition/0.1 of oil at 100 mg/mL, as compared to positive controls, Ascorbic acid and BHT at 1 mg/mL with percentage of inhibition 75 and 75.6 respectively. Illustration was displayed in Figure 15.

Discussion

Phytochemicals are invaluable sources of raw material for both traditional and orthodox medicine. Seed-oils containing bioactive agents such as alkaloids, tannins and phenolics thus, readily present

themselves as a good source of raw material in modern and traditional medicine. Phytochemical analysis of *Ceiba pentandra* seed oil indicates that all the above mentioned phytochemicals are present in the seed oil sample. Phenolic compounds are a class of antioxidant agents which act as free radical terminators and also involved in retardation of oxidative degradation of lipids [14]. In addition, Odukoya et al. [15] has reported a strong relationship between phenolic content and antioxidant activity in selected fruits and vegetables. Thus, the presence of phenolic compounds in *Ceiba pentandra* seed oil is an added value to its nutritional and health potential. Furthermore, the occurrence of flavonoids in the *Ceiba pentandra* seed oil which are also phenolic compounds, similarly improves the economic and health potential of the oil. This is in agreement with previous findings which suggested that flavonoids carry out antioxidant action through scavenging or chelating process and are reported to play a preventive role in cancer and heart disease [16]. Alkaloids and their synthetic derivatives are being used as basic therapeutic agents for their analgesic, antispasmodic and bactericidal effects. However alkaloids and flavonoids inhibit certain mammalian enzymatic activities such as those of phosphodiesterase,

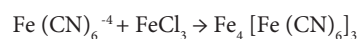
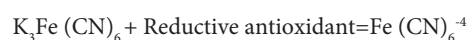
prolonging the action of cyclic-AMP. Alkaloids also affect glucagons and thyroid stimulating hormones [17].

Tannins have shown potential antiviral, antibacterial and antiparasitic effects [18-20]. When incubated with red grape juice and red wines with a high content of condensed tannins, the poliovirus, herpes simplex virus, and various enteric viruses are inactivated [21]. It is believed that tannins isolated from the stem bark of *Myracrodruon urundeuva* may have neuroprotective functions capable of reversing 6-hydroxydopamine-induced toxicity. The plant has shown promise as a potential therapeutic agent, which may be beneficial in patients with neurological disease [22]. Souza et al. discovered that the tannins isolated from the stem bark also have anti-inflammatory and antiulcer activity in rodents, showing a strong antioxidant property with possible therapeutic applications [23]. Foods rich in tannins can be used in the treatment of HFE hereditary hemochromatosis, a hereditary disease characterized by excessive absorption of dietary iron, resulting in a pathological increase in total body iron stores. Tannins can also be effective in protecting the kidneys.

The antioxidant capacities of the compounds and of volatile oils show concentration dependence, because of the solubility problems the reducing capacity of components could be measured in a lower concentration range as seen in graphical versions. Moreover, the antioxidant potential of *Ceiba pentandra* seed oil was further highlighted by the quenching of DPPH free radicals, which is a proton free radical commonly used to determine the free radical scavenging power of antioxidants [24]. The decrease in absorbance of DPPH-oil mixture in this study, which measured the extent of radical scavenging potential of the oils supported the findings of [25]. In addition, reducing power of a compound is related to electron transfer ability of the compound which could lead to the neutralization of free radical [26]. The observed increase in reducing power of the oils which was concentration dependent suggested that they are good electron donors. Studies have shown that the reducing power capacity of a compound may serve as a significant indicator of its potential antioxidant activity [27]. This suggestion was confirmed in this present study where by *Ceiba pentandra* seed oil with more phenolic content exhibited greater antioxidant activity.

The total antioxidant potential of the oil was determined using a ferric reducing ability (FRAP) assay. The FRAP assay is based on the ability of a sample to reduce Fe^{3+} in a Tripyridyltriazine (TPTZ) solution to Fe^{2+} and create the blue-colored complex Fe^{2+} -TPTZ. Increased concentrations of the above complex means an increased FRAP value.

Antioxidant compounds cause the reduction of ferric (Fe^{3+}) form to the ferrous (Fe^{2+}) form because of their reductive capabilities. Prussian blue colored complex is formed by adding FeCl_3 to the ferrous (Fe^{2+}) form. Therefore, reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm [28,29]. In this assay, yellow color of the test solution changes to green or blue color depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric reducing power.



Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action [30,31].

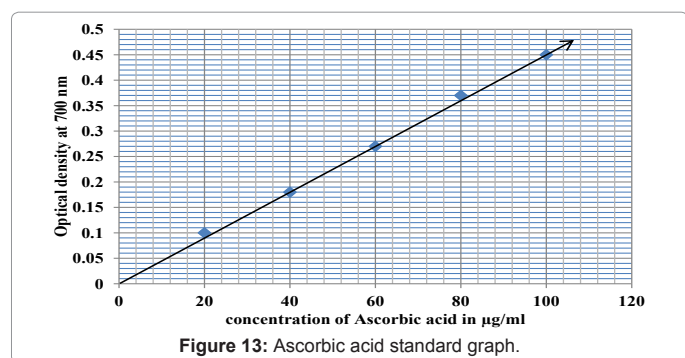


Figure 13: Ascorbic acid standard graph.

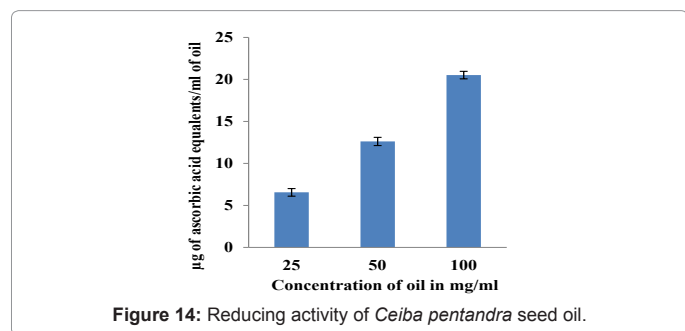


Figure 14: Reducing activity of *Ceiba pentandra* seed oil.

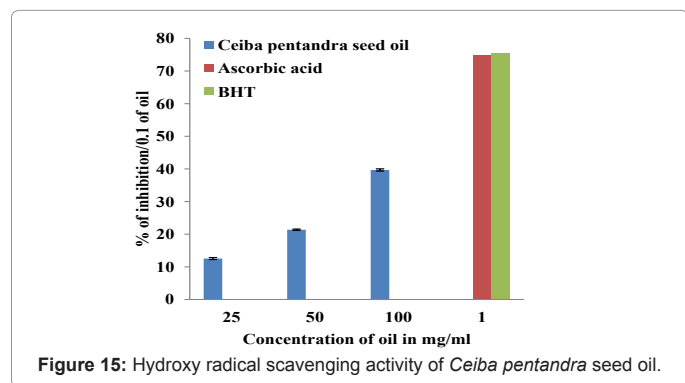


Figure 15: Hydroxy radical scavenging activity of *Ceiba pentandra* seed oil.

Hydroxyl radicals are one of the quick initiators of the lipid peroxidation process by abstracting hydrogen atom from unsaturated fatty acids or simply auto oxidation of polyunsaturated fatty acids, found primarily in membranes. Reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species [32,33]. Nevertheless, the *Ceiba pentandra* seed oil exhibited remarkable phytochemical and antioxidant properties which need to be explored for economic, nutritional and health applications.

Conclusion

Seed oils from the plants have been attributed for their nutritional, industrial and medicinal values. This study highlights phytochemical composition and antioxidant activities of the seed oil from the *Ceiba pentandra*. Therefore, the importance of the phytochemical constituents and antioxidant activities of oil in the maintenance of health is strengthened as trend of the future is moving towards using seed oil as medicine in the management of various chronic diseases.

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