



## A Study on Bioactive Compounds of Ceiba Seeds

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### ABSTRACT

The Ceiba seed powders were tested for its phytoconstituents like carbohydrate, alkaloid, glycoside, tannin, protein, phenolic compound, fixed oil, steroid qualitatively. The carbohydrate, protein, amino acids were assessed quantitatively likewise fluorescence, percent yield, and behavior of Ceiba seed powder with different chemical reagents was also performed. The results were found to be positive for the phytochemicals tested qualitatively. The carbohydrate content was found to be  $62.66 \pm 2.30$  mg/g, total protein content was  $16.17 \pm 0.15$  mg/g protein, amino acid content calculated was  $03.86 \pm 0.57$  mg/g. The behavior of the seed powder with different chemical reagents showed positive results for alkaloid, steroid, anthraquinone, protein, and flavonoid. Green color fluorescence was observed in fluorescence study. The results obtained showed that it contained potent phyto-compounds which find application in drug industry. The recovery percent obtained from 15g Ceiba seed powder was 23.60.

**Keywords:** Aqueous, Ceiba, Extract, Nutrients, Phytochemicals.

### INTRODUCTION

Ceiba tree is an evergreen tree belonging to Malvaceae family. It is native to South America, now spread to West Africa and South East Asian rainforests of the Malay Peninsula and Indonesian archipelago. It is a national tree of Guatemala and sacred world tree of Malayan civilization. The white, fluffy seed covering is used in pillows, mattresses. Ceiba seeds are buoyant, water resistant, elastic in nature, which find its use in padding<sup>1</sup> and floating devices as the silky fibers that disperse the seeds are too small for weaving. Similar to pulses, oil seeds are richest source of protein and contain high level of fat providing energy. West, Central Africa and North America people use Ceiba spp. as anti-inflammatory, analgesic, anti-bacteria, anti-cancer, anti-diabetic, anti-fungal, anti-malarial, anti-oxidant,<sup>2,3</sup> antipyretic<sup>4,5</sup> and wound healing.<sup>6</sup> Other parts such as seed, leaves, bark and resin are used to treat dysentery, fever, asthma and kidney diseases. In Nigeria, the leaves are cooked in the form of slurry sauce comparable to okra. The oil was antibacterial and antifungal in nature. Considering the benefits of Ceiba seeds, it was decided to study preliminary aspects with respect to Ceiba seed, a seed of commercial importance.

### MATERIALS AND METHODS

#### Sample collection

The sample-dry Ceiba seeds were purchased from shops at Krishnagiri, Krishnagiri District, Tamil Nadu, India. The purchased seeds were cleaned thoroughly and ground to powder for further use.

#### Aqueous extract preparation

Aqueous extract was prepared by dissolving 15g of Ceiba seed powder in 200ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30-40°C for 20minutes. Then the water extract was filtered through filter paper. The filtrate was kept in a beaker and allowed to dry by heating in a boiling water bath. The gummy residue obtained was used for the analysis of percentage yield, and the remaining marc left was extracted with water and used for Qualitative analysis.

#### Phytochemical analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures<sup>7,8</sup> fluorescence analysis,<sup>9</sup> behavior of drugs powder with different chemical reagents.<sup>10</sup>

#### Test for carbohydrate

**Molisch's test:** To the extract added few drops of alcoholic alpha naphthol solution, few drops of concentrated Sulphuric acid along the sides of test tube. Positive result gives purple or violet colored ring at the junction.

**Fehlings test:** To the extract added equal amount of Fehling's A and B solution, heat the tubes in a boiling water bath. Brick red precipitation of cuprous oxide is formed, if reducing sugar is present.

**Benedicts test:** To the extract add Benedict's reagent, the tubes were heated in a boiling water bath. Red precipitation indicates positive result.



**Test for alkaloids**

Wagners test: To the extract add few drops of iodine solution in potassium iodide. Reddish brown precipitate shows positive result.

Hagers test: To the extract add few drops of saturated solution of picric acid. Yellow color precipitation signifies positive result.

**Test for steroids and sterols**

Liebermann-Burchard test: To the extract add 2ml chloroform, 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid. Bluish red to cherry red color in chloroform layer shows positive result.

Salvoski test: To the extract add few drops of chloroform, concentrated sulphuric acid. Bluish red to cherry red color.

**Test for Glycosides**

Legal test: To the extract added pyridine, sodium nitroprusside. Positive result shows pink red color.

Baljet test: To the extract add picric acid. Appearance of orange color signifies positive result.

**Test for Saponins**

Foaming test: Foams produces when the extract is mixed with water.

**Test for flavonoids**

Shinoda test: To the extract added magnesium turnings, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicates positive result.

Zinc hydrochloride test: To the extract added zinc dust, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicates positive result.

**Test for tannin and phenolic compounds**

Ferric chloride test: To the extract add ferric chloride. Formation of greenish black color shows positive result.

Potassium dichromate test: To the extract add potassium dichromate solution. Positive result is confirmed by a formation of brown precipitate.

Gelatin test: To the extract add 1% gelatin solution containing 10% sodium chloride gives white precipitation.

**Test for protein and amino acids**

Biuret test: To the extract added 4% sodium hydroxide, few drops of 15% copper sulphate gives purple color.

Ninhydrin test: Bluish violet color forms when a solution of ninhydrin and extract mixture was heated.

Heat test: Protein coagulation shows positive result when test solution is heated on a boiling water bath.

**Test for fixed oil**

Copper sulphate test: Blue color forms when the extract is mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

**Quantitative analysis of phytonutrients**

Total carbohydrates,<sup>11</sup> proteins,<sup>12</sup> aminoacids<sup>13</sup> were performed according to the standard prescribed methods.

**Estimation of carbohydrate**

The total carbohydrate was estimated by anthrone method. 1mg of Ceiba seed powder was hydrolyzed to simple sugars by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cool to room temperature. After neutralizing, the contents were centrifuged and 0.1 ml of supernatant was used for the analysis. To the sample add 4ml of anthrone reagent and contents were heated in a boiling water bath for 8 minutes. The tubes were cooled and read at 630nm using spectrophotometer Shimadzu Model - UV 1800. The standards were developed with glucose. Standard graph plotted was used to find out the concentration of glucose present in unknown/sample.

**Estimation of Protein**

The total protein was estimated by Lowry's method. To 0.1ml of extract added 2ml of alkaline copper reagent, mixed well and incubated for 10minutes. After the incubation period 0.2ml of folin ciocalteau reagent (diluted in the ratio of 1: 2) was added and allowed for 30minutes incubation, then read at 660nm using spectrophotometer Shimadzu - Model UV 1800. The standards were developed with bovine serum albumin. Standard graph plotted was used to find out concentration of protein present in unknown/sample.

**Estimation of Amino acids**

The amino acid was estimated by Ninhydrin method. To 0.1 ml of sample added 1ml of Ninhydrin solution dissolved in ethanol. Cover the test tube with a piece of paraffin film to avoid the loss of solvent due to evaporation. With gentle stirring, react at 80-100°C for 4-7 minutes. Cool the test tubes and the color developed was read at 570nm. Tyrosine was used for developing standards.

**Statistical Tool**

Each experiment was carried out in triplicate and the results are given as the mean  $\pm$  standard deviation. The Mean and Standard deviation (S) was calculated by using the following formula: Mean = Sum of x values / n (Number of values),  $s = \frac{\sqrt{\sum(x-M)^2}}{n-1}$

**RESULTS AND DISCUSSION**

The percentage recovery of the aqueous extract obtained was calculated and expressed in Table 1.



**Table 1:** Percentage yield of Ceiba seed aqueous extract

Name of the seed	Weight taken for extraction	Initial weight of beaker(gm)	Final weight of beaker(gm)	Weight of the extract Powder(gm)	% recovery
Ceiba seed powder	15g in 200ml water	147.1558	150.6968	3.541	23.60

The extract obtained after drying was 3.541gm and its yield in percent was found to be 23.60. (Table 1)

#### Analysis of seed powder for its behavior

**Table 2:** Behavior of Ceiba seed powder with different chemical reagents

Tests	Observation	Inference
Powder + Picric acid	Yellow color	Presence of alkaloid
Powder + Conc. H <sub>2</sub> SO <sub>4</sub>	Reddish brown color	Presence of steroids
Powder + Iodine solution	Pale brown color	Absence of starch
Powder+ Aqueous 5% KOH	Pale brown color	Presence of anthroquinone
Powder + NaOH	Yellow color	Presence of flavonoid
Powder+ Aqueous AgNO <sub>3</sub>	White precipitate	Presence of protein

**Table 3:** Phytochemicals present in Aqueous Ceiba seed extract

Name of the test	Results
<b>Test for carbohydrate</b>	
a) Molisch's test	+++
b) Fehlings test	+++
c) Benedicts test	++
<b>Test for alkaloids</b>	
a) Wagners test	+++
b) Hagers test	++
<b>Test for steroids and sterols</b>	
a) Libermann - Burchard test	+
b) Salwoski test	+
<b>Test for Glycosides</b>	
a) Legal test	++
b) Baljet test	+++
<b>Test for saponins</b>	
Saponin test	+
<b>Test for flavonoids</b>	
a) Shinoda test	+
b) Zinc hydrochloride test	-
<b>Test for tannin and phenolic compounds</b>	
a) Ferric chloride test	++
b) Potassium dichromate test	++
c) Gelatin test	-
<b>Test for protein and amino acids</b>	
a) Biuret test	++
b) Ninhydrin test	+++
<b>Test for fixed oil</b>	
a) Copper sulphate test	+++

+ Slight changes, ++ Moderate, +++ Stronger reactions

The behavior of seed powder with different chemical reagent showed positive result for alkaloid, steroid, anthraquinone, protein and flavonoid. (Table 2)

#### Fluorescence Analysis

Fluorescence analysis of Ceiba seed extract when observed under day light was pale brown in color but when seen under UV light it was found to fluoresce green in color.

#### Phytochemical analysis

The results of phytochemical analysis are shown in Table 3.

The results of qualitative analysis showed that it contains carbohydrate, alkaloids, steroid, sterols, glycosides, saponins, flavonoids, tannins, phenolic compounds, protein, amino acids and fixed oil. (Table 2)

#### Phytonutrients Analysis

The phytonutrients estimated were tabulated in Table 4.

**Table 4:** Phytonutrients in Aqueous extract of Ceiba seed

Phytonutrients	Calculated nutrient content (mg/g)
Total carbohydrate	62.66 ±2.30
Total protein	16.17 ±0.15
Amino acids	03.86±0.57

Values are Mean ± SD for three experiments

The content of carbohydrate was found to be higher, showing 62.66±2.30mg/g glucose, whereas, the total protein was 16.17±0.15mg/g albumin, and amino acid was 03.86±0.57mg/g tyrosine. In the present study, the nutrient levels were found to increase in the following order: Amino acids < Total protein < Total carbohydrate. The Ceiba seeds was studied by Krishnaveni et.al for its phytoconstituents, fatty acids through GC-MS as well as its antimicrobial,<sup>14</sup> antioxidant activities.<sup>15</sup>

#### CONCLUSION

This study concludes that it contains carbohydrate, protein, amino acids both qualitatively and quantitatively. The fluorescence study revealed green fluorescence and the recovery percent was 23.60. The study confirms it to be a natural source for drug preparation with more therapeutic potential.

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