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"Vitamins and Antioxidants Activity of *Cassia Siamea Lam* Seed's Oil"

Neetu, Roli Agrawal

Research Scholar, Associate Professor

Department of Chemistry,

Shri Varshney College Aligarh-202001,

Dr. Bhim Rao Ambedkar University, Paliwal Park, Agra (U.P.) - 282004, India

neetijhansi@gmail.com

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KEYWORDS

Cassia Siamea Lam, Seed oil, Antioxidant activity, Vitamins B1, B2, B3 and Vitamin C.

ABSTRACT:

A medicinal plant from the family- Fabaceae, subfamily- Caesalpiniaceae called Cassia Siamea Lam has been chosen for study. In this study, we determined the Antioxidant activity, and Vitamins of *Cassia Siamea Lam* seed oil. The oil from the seeds of *Cassia Siamea Lam* was extracted with a Soxhlet extractor using petroleum ether as a solvent. Antioxidant activity was defined by a Spectrophotometer using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) solvent. Vitamins B1, B2, B3, and Vitamin C were determined by the HPLC technic. The Antioxidant activity of *Cassia Siamea Lam* seed oil is 7.5mg TE/100g. This oil contains Below Detection Limit of vitamins B1, B2, and B3, as well as 15.70 mg/kg of vitamin C. Vitamin C is essential for a number of body functions, including collagen formation, iron absorption, immune system function, wound healing, and the preservation of cartilage, bones, and teeth. The body needs Vitamins to boost the immune system and speed up wound healing. As an Antioxidant, it acts to shield your cells from free radicals, which have been linked to cancer, heart disease, and other illnesses. This study suggests that *Cassia Siamea Lam* seed oil is rich in Antioxidants and Vitamin C.

1. Introduction

Oils and fats derived from seeds and nuts are typically considered vital components of a human diet. The primary nutrients needed by the human body are fats and oils, along with proteins, carbs, vitamins, and minerals. Vegetable oils are primarily essential because of their nutritional value. Vegetable oils made from plant seeds are used for a variety of industrial applications as well as food and other necessities, and they have been playing a significant role in improving human comfort in many parts of life [11]. Concerns concerning the use of vegetable oils as a material source instead of mineral or petroleum oil have grown over the past few decades. Mineral oil is a serious environmental hazard, which is the primary cause of this worry [18]. Oil seed rape is a particularly important crop since the seed is usually 42% oil. Hemp seed oil also offers a sufficient amount of antioxidants (Vitamin E). Carotene is a precursor to Vitamin A. [22, 23, and 24].

Vitamins B and Vitamin C are among the Vitamins that are water-soluble. Thiamin, often known as Vitamin B1, is a member of the Vitamin B complex family. The human body depends on Vitamin B1 for wellness. It is involved in the process of turning lipids and carbohydrates into energy [14]. Some important enzymes involved in the metabolism of carbohydrates use Vitamin B1 as a co-enzyme precursor [3]. Additionally, it aids in the structural growth of brain cells [7]. Riboflavin is another name for Vitamin B2. It contributes to the metabolism of energy. Glutathione, the most important Antioxidant that guards the body against free radicals, is recycled by Vitamin B2. Additionally, it supports iron metabolism, and as iron is necessary for the development of red blood cells, a lack of it increases the risk of anemia [21].

Niacin is another name for Vitamin B3. A class of substances known as niacin has Vitamin-like properties. Nicotinic acid, nicotinamide, and various enzymatic forms make up Vitamin B3 [17]. The synthesis of energy from dietary proteins, carbs, and lipids is principally mediated by the two different forms of Vitamin B3 known as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) [16]. Enzymes that contain NAD, NADP, and niacin scavenge free radicals and guard tissues from oxidative damage [15].Red blood cell synthesis, glucose metabolism, liver detoxification, brain and nervous system function are all impacted by Vitamin B6 [6]. Ascorbic acid, also known as Vitamin C, is a popular dietary supplement [5].

Antioxidant properties of Vitamin C can shield cellular structures from the damaging effects of free radicals. Its

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important role in iron absorption is to change iron into a form that the intestines can easily absorb. Collagen, a substance used in the structure of the human body, is produced only when Vitamin C is present. Vitamin C is also necessary for the manufacture of several neurotransmitters, particularly those involved in the signaling of emotions, ideas, and directives throughout the brain and nervous system. Numerous bodily processes, including the production of collagen, iron absorption, healthy immune system operation, wound healing, and preservation of cartilage, bones, and teeth, depend on Vitamin C. Vitamin C has been shown in a few clinical tests to reduce wrinkles. [4]

The phytonutrients, minerals, Vitamins, and Antioxidants found in mustard seeds are truly very abundant. Niacin, thiamin, riboflavin, pyridoxine (Vitamin B-6), and pantothenic acid are just a few of the vital B-complex Vitamins that can be found in abundance in mustard greens. These Vitamins are necessary in that the body needs to replace them from outside sources. These B complex Vitamin subgroups support nervous system health, metabolic control, and enzyme synthesis. It is thought that applying mustard oil to the scalp may encourage hair growth. [25, 26, and 27].

Antioxidant substances are currently gaining importance due to their use in food product creation to preserve color, flavor, and shelf life. Oxidative rancidity is a common cause of quality changes in many food items that contain lipids. Rancidity in food items can be caused by a variety of factors, such as how the food is prepared and stored, the type of lipids used chemical alterations, and more [13]. Synthetic Antioxidants like butyrated hydroxyl toluene (BHT) and butyrated hydroxyl anisole can be added to food to avoid lipid oxidation (BHA). However, owing to their potential to cause cancer in humans, these Antioxidants have recently been restricted [28, 10]. Natural Antioxidants like those in olive oil, cranberries, oil seed, etc. are generally preferred by consumers [12]. Finding novel natural Antioxidants that are safe for consumers is therefore of increasing interest to researchers [8, 20]. Numerous herbs have been investigated, including sage, oregano, and rosemary. It was discovered that these herbs had high Antioxidant activities [30], which can be used in cosmetics, food products, and other things [19].

Cassia Siamea Lam, a medicinal plant belonging to the Caesalpiniaceae subfamily of the Fabaceae family. *Cassia Siamea Lam* is a roughly 18 m long evergreen tree with a straight trunk that can reach a diameter of 30 centimeters and grey to light brown bark. Large, erect, bright yellow flowers that are 20 to 30 centimeters long and 13 centimeters broad growing in bunch at the ends of twigs. The seeds of *Cassia Siamea Lam* are lustrous, dark brown, bean-shaped, and up to 8mm long [9, 29]. This study determined the antioxidant activity and vitamin contents of *Cassia Siamea Lam* seed oil, together with the B and C vitamins..

2. Materials and Method

Collections of plant material - *Cassia Siamea Lam's* seeds were collected in Dhanipur Mandi Aligarh. The collected seeds were dried in direct sunlight for one to two days, then ground into a fine powder and stored in an airtight container for chemical analysis.

Oil extraction- The oil was extracted from *Cassia Siamea Lam* seed flour using a solvent extraction process that employed petroleum ether as a solvent. [7, 2]. 100 g of powdered *Cassia Siamea Lam* seeds were placed in filter paper and put inside the Soxhlet extractor together with petroleum ether, which served as the extraction solvent. After 24 hours, the solvent was recovered using a water condenser, and the remaining oil was transferred into a desiccator and stored in an airtight container until analysis.

Determination of Vitamins B1, B2, B3, and B6-

Solvents and Reagents- Riboflavin (B2),Thiamin (B1), Niacin amide(B3), Pyridoxine(B6), Distal Water, Syringe Filter (0.45µm pore size), PVDF Filter Membrane (0.45 or 0.22µm pore size), Methanol(HPLC Grade),10% Acetic Acid.

Sample preparation – Weigh 1-2gm sample add 5ml diluent mix the sample by manual shaking, Vortex volume makeup 10 ml centrifuge the sample at 5000rps for 10 min than filter the sample with syringe filter and inject in HPLC.

Quantification of Vitamins B1, B2, B3, and B6 – Vitamins B1, B2, B3, and B6 were analyzed using a modified method. Vitamins were evaluated using high-performance liquid chromatography (HPLC) with PDA Detector. HPLC was capable of pressures up to 3000 psi with an injector qualified of 100 µL injections Operating condition was eluent flow rat 2.0±0.2ml/min; the temperature ambient Detector was Capable of measuring absorbance at 280 nm, with sensitivity 0.02AUFS. Precolumn set at 2mm id×2cm stainless steel, packed with 40µm pellicular reversed-phase. A column set at C-18(250mm×4.6mm, 5µm) and Mobile Phase A consisted of 1.4g n-hexane Sulphonic Acid Sodium Salt: MeOH (75:35), Ratio of Mobile Phase was 100%(A).Degas 2-5 min under vacuum the injection volume 20µ L and Flow Rate 1ml/min. The

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data were integrated and analyzed using the Chromatography Laboratory Automated Software system. The amount of Vitamins in oil was calculated as mg/kg external calibration curves (R2=1), obtained for vitamins standard. This formula calculated all Vitamin values.

$$\text{Con. of Analyte (mg/kg)} = \frac{\text{Peak area of Sample} \times \text{Std. Conc. (mg/kg) Sample volume (ml)} \times \text{Dilution}}{\text{Peak area of standard} \times \text{Sample Weight (g)}}$$

$Y = mX + C$

Where, Y= Analyte area

X= Concentration of Analyte

m = slope of the calibration curve

C =Y-axis intercept value

Determination of Vitamins C

Solvents and Reagents- Standard Ascorbic acid, Milli-Q Water, Acetic Acid, HPLC Column-C 18 (250mm ×4.6mm×5µm) and UPLC Column-C 18(150mm×4.6mm×2.7µm)

Sample preparation-

(i)Stock solution of Vitamin C-Weight 10 mg of Ascorbic Acid Standard into 10 ml amber color volumetric flask and dissolved in 10 ml Mobile Phase. Stability of stock solution is 3 month store at 4^0C.

(ii)Intermediate Standard (100)- Pipette 1ml of Ascorbic Acid into 10ml volumetric flask and makeup volume with mobile Phase.

(iii)Calibration Standard-Prepare a set of calibration standard ranging from 1ppm-100ppm by using Mobile Phase as diluents.

(iv) Mobile Phase Preparation- Take Milli-Q water in 1000ml Measuring Cylinder and Add 50 ml Acetic Acid Mix Properly than makeup volume 1000ml with Milli- Q water.

Quantification of Vitamins C- Vitamin C was evaluated using a modified method of high-performance liquid chromatography (HPLC). Weighted 0.2-2±0.1gm sample in 50 ml Centrifuge tube and added 10 ml mobile Phase and mixed it and shaken for 5 min. and Centrifuged at 5000 rpm for 5 min. Take Supernatant was filtered by using a PVDF syringe filter and it was injected in HPLC for determination. Precolumn was set at 2mm id×2cm stainless steel, packed with 40µm pellicular reversed-phase. The column set at C-18(250mm×4.6mm,5 4.6mm, 5µm) and Mobile Phase A consisted of a 5% Acetic Acid Ratio of Mobile Phase was 100% and the Flow rate consists of 1ml/min. The column temperature was set at 350C. Degas 2-5 min under vacuum the injection volume was 20µL and the running time was 10 min. Post run time was 2 min and Mode was isocratic. The data were integrated and analyzed using the Chromatography Laboratory Automated Software system. The amount of Vitamin C in oil was calculated as mg/kg external calibration curves (R2=1), obtained for vitamins standard. This formula calculated vitamin C-

$$\text{Con. of Analyte (mg/kg)} = \frac{\text{Peak area of Sample} \times \text{Std. Conc. (mg/kg) Sample volume (ml)} \times \text{Dilution}}{\text{Peak area of standard} \times \text{Sample Weight (g)}}$$

$Y = mX + C$

Where, Y= Analyte area

X= Concentration of Analyte

m = slope of the calibration curve

C =Y-axis intercept value

Determination of Antioxidant activities

Solvents and Reagents- DPPH (2, 2-Diphenyl-1-picrylhydrazyl) used as a reagent solution, Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) used as a standard solution, Methanol, and Deionized water.

Sample preparation – (i) preparation of reagent- 40 grams of DPPH was taken in a 2-liter volumetric flask and 1 liter of HPLC grade methanol was added. The solution was covered with an aluminum foil to protect the light when all DTPH was completely dissolved then 1 liter of distilled water was added to the solution.(i) preparation of standard- 50.00 ± 0.1 milligrams of Trolox was taken into a 100 mL volumetric flask and 50 mL HPLC grade methanol was added. The flask was covered with aluminum foil to protect the solution from light. The solution was put on a magnetic stirrer until dissolved. After it was dissolved then 50 mL DI water was added. Invert 13 times and it was filled to volume with HPLC-grade methanol.

Quantification of Antioxidant activities- Determination of the Antioxidant activity of the sample was carried out according to the method of (1). The sample and standard solution of A, B, C, D, and E were marked with different weights and concentrations of methanol solutions were prepared to add 50 ml of DPPH solution and mixed. The reaction mixture was incubated in an orbital shaker at 35 ± 2°C for 4 h at 250 rpm. The discoloration was determined at 517 nm by spectrophotometer. The percentage DPPH of inhibition (I %) was calculated using the following equation: I% = [(Control abs – Sample abs) × 100] / Control abs, and Trolox equivalent Antioxidant capacity mg/100g = [IC50 Trolox ×100] / [IC50 Sample×1000]

3. Results

Figure-1: HPLC-Graph of Vitamin B1 on Area and Amount

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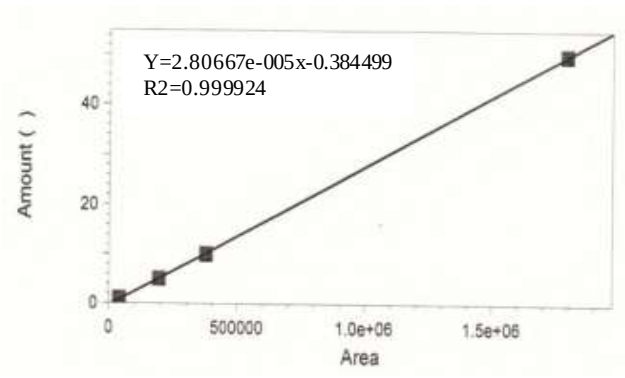

Figure-2: HPLC-Graph of Vitamin B2 on Area and Amount

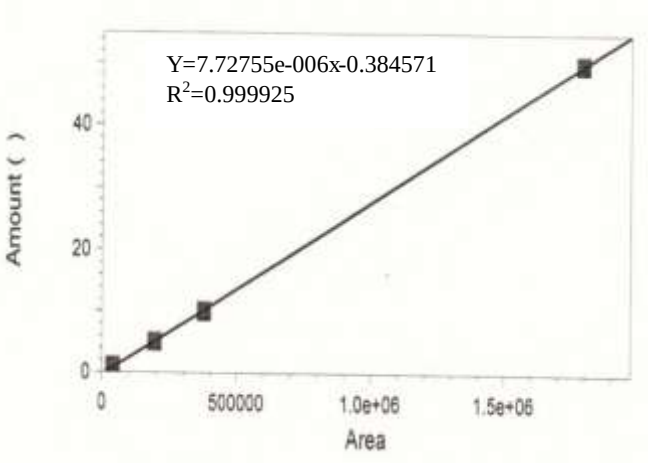

Figure-3: HPLC-Graph of Vitamin B3 on Area and Amount

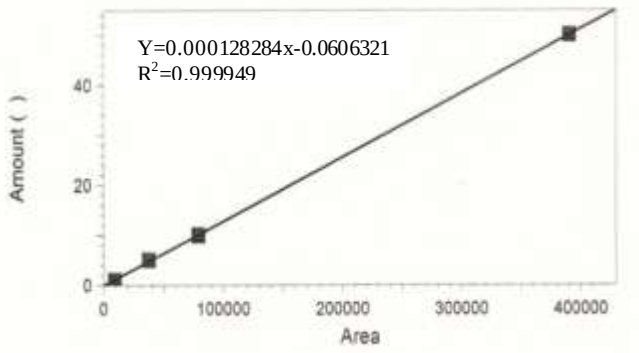

Figure-4: HPLC-Graph of Vitamin B6 on Area and Amount

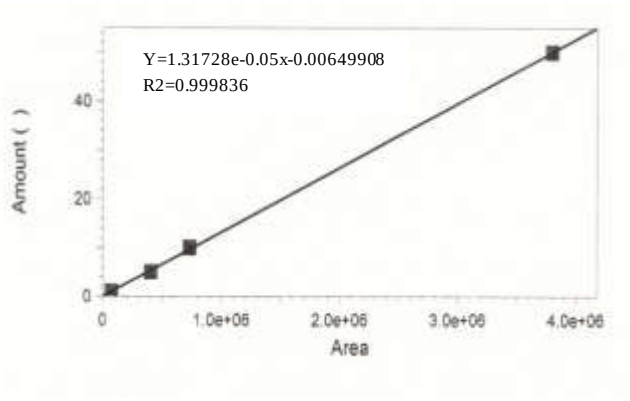

Figure-5: HPLC-Graph of Vitamin C on Area and Amount

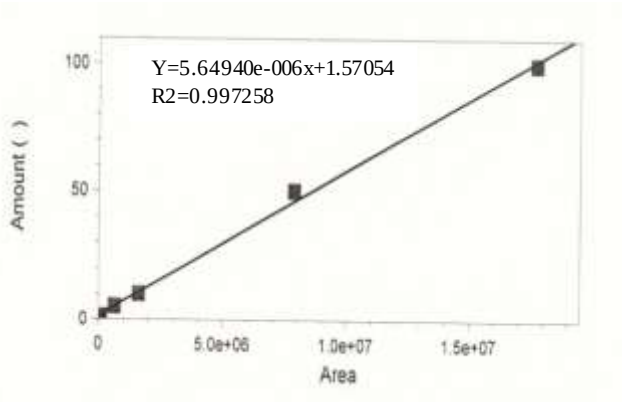

Figure-1, 2, 3, 4, and 5 show the Graph between Amount (ml) and Area of Sample for Vitamins B1, B2, B3, B6, and Vitamin C. The Limit of Quantification of Vitamins B1, B2, and B3 were 0.25mg/100kg and the calculated value of these Vitamins in *Cassia Siamea Lam* seed oil was Below the Detection Limit. The Limit of Quantification of Vitamin C was 5 mg/kg and the calculated value of Vitamin C was 15.70mg/kg. Numerous bodily processes, including the production of collagen, iron absorption, immune system operation, wound healing, and maintenance of cartilage, bones, and teeth all depend on Vitamin C. Vitamins are required by the body to strengthen the immune system and hasten wound healing. In a few clinical studies, Vitamin C was found to lessen wrinkles. According to this study, the seed oil from *Cassia Siamea Lam* is high in Vitamin C.

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Figure-6: Chromatogram of Vitamin B1, B2, B3, and B6-

Figure-6 represents the Chromatogram of Vitamin B1, B2, B3, and B6. The value of Vitamins B1, B2, and B3 was Below the Detection Limit. These chromatograms indicate that this oil contains less Vitamins B1, B2, B3, and B6.

Figure-7: Chromatogram of Vitamin C-

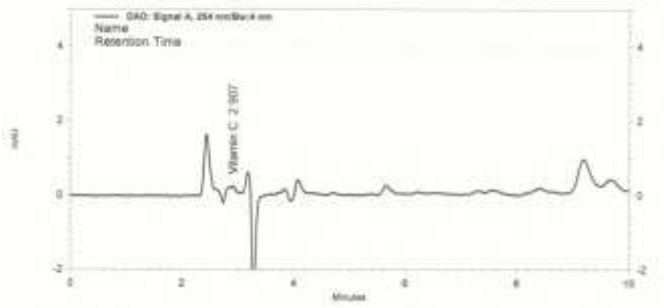

Figure-7 represents the Chromatogram of Vitamin C. The value of Vitamin C was 15.70mg/kg. These chromatograms indicate that this oil contains greater Vitamin C.

Capacity of Antioxidant –

Figure-8: Graph between Conc. And IR Trolox for *Cassia Siamea Lam* seed oil

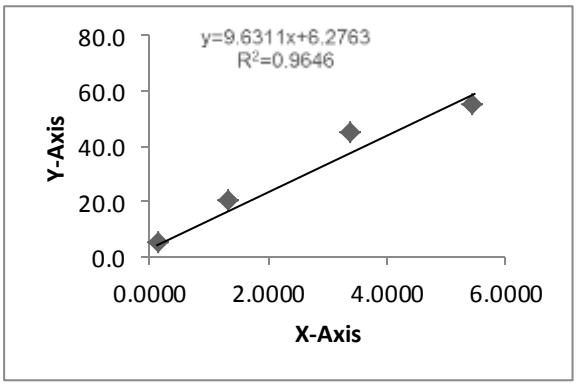

Figure-8 shows the graph between the concentration and Absorbance graph of *Cassia Siamea Lam* this figure indicates that the value of the IC50 sample is 4.5 g/ml and the standard IC50 Trolex value is 336.10 µg/ml. The calculated value of Trolox equivalent antioxidant capacity value mg/100g = 336.10x100/4.5x1000 = 7.5mg/100g. *Cassia Siamea Lam* seed oil The Antioxidant capacity content of the *Cassia Siamea Lam* oil was 7.5 mg/100g. This demonstrates that this oil will provide a superior source of Antioxidants. It functions as an Antioxidant to protect your cells from free radicals, which have been associated with cancer, heart disease, and other disorders. According to this study, the oil from *Cassia Siamea Lam* seeds is high in Antioxidant

4. Conclusion

The current experiment will be useful in gathering data regarding the Antioxidants and Vitamins in *Cassia Siamea Lam* seed oil and in examining the several possibilities. The oil extracted from the seed of *Cassia Siamea Lam* is a good source of Vitamin C and Antioxidants so it could be seen as an Antioxidant to protect your cells from free radicals, which have been linked to cancer, heart disease, and other disorders. Vitamin C was found to lessen skin wrinkles so this oil may be beneficial for treating wrinkles.

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