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# Antioxidant Studies and Characterization of *Cassia tora* (Sickle Senna) Seeds Oil

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## ABSTRACT

*Cassia tora* seeds were utilized in ancient healthcare systems to cure numerous symptoms and disorders. Recently, there has been widespread information about the potency of the seed to cure even eye ailments like glaucoma. However, there is no systematic scientific backing or research explaining the detailed character of the seeds and their oil; as such investigation into its properties is required now more than ever. N-hexane, ethyl acetate, and distilled water were used to extract the oil from fresh Cassia tora seeds. The extracted oil's antioxidant potential was evaluated, and scavenging activity investigations were conducted. The results revealed an interesting antioxidant activity of the oil compared to standard antioxidants, signifying its potential in various applications and products. The FTIR spectroscopy confirmed the presence of hydroxyl, esters, aliphatic hydrocarbon, carbonyls, aldehyde, and unsaturated bonds which are characteristic of fatty acids and triglycerides. The saponification value of 187.71 mg KOH/g, an iodine value of 7.64 mg/g, and an acid value of 1.19 mg/g were obtained. The numerous results obtained in this study reinforced the potential of Cassia tora oil in various applications, including its potential to be employed as an antioxidant or part of formulations of various effective antioxidants.

**Keywords:** *Cassia tora*, Extraction, Characterization, Antioxidant Studies.

## INTRODUCTION

Plant-derived medicines have been used in traditional healthcare systems for the treatment of various ailments and diseases since time immemorial (Firdaus et al., 2025). According to the World Health Organisation (WHO), it has been estimated that 80% of the world's population is still reliant on traditional remedies to keep their health and combat various diseases (Davis & Choisy, 2024) . *Cassia tora Linn*. also known as sickle senna (English common name is a wild plant species in the family Leguminosae, indigenous to Central America. In East Asia, whole plants, seeds, leaves, and roots of sickle senna are commonly employed in traditional medicine. Its seeds can be eaten while the roasted ones can be used to replace coffee. Similarly, *Cassia tora* tea is utilized in Korea to renew human vision (Islam et al., 2023).

In addition, it has been found to cure arthritis, snakebites, oedema, and plethora of skin problems like leprosy, ringworm, itching, and psoriasis (Osunga et al., 2023) . Furthermore, Cassia tora seeds exhibit antidiabetic, antifungal, antihepatotoxic, antimutagenic, antigenotoxic, and larvicidal properties (Islam et al., 2023) . Cassia tora seeds also contain chrysophanic acid, which is used to heal wounds, skin problems (eczema, ringworms, and scabies), gastrointestinal disorders (uterine disorders and ulcers), jaundice, rheumatism, and anorexia (Khurm et al., 2021).

Cassia tora has been demonstrated to have curative action against a variety of medicinal applications in the traditional system of medicine, including bronchitis, constipation, conjunctivitis, ulcer, hypertension, hypercholesterolaemia, heart disease, fungal infections, diabetes, oedema, glaucoma, nyctalopia, ringworm, dermatological conditions, and plaque. Similarly, extracts of different parts of Cassia tora have been used as analgesic, anticonvulsant, antipyretic, antibacterial, antifungal, anthelmintics,

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diuretic, expectorant, laxative, purgative, and useful in treatment of glaucoma, hypertension, skin disease, ringworm, leprosy, flatulence, colic, dyspepsia, constipation, cough, and itch (Ramakrishnan et al., 2017).

Despite its several potential, there are limited systematic studies on the characterizations and antioxidant studies of Cassia tora seed oil. However, Ramakirishnan *et al* used the steam distillation method to extract oil from Cassia tora seeds to determine its nutritional and antioxidant properties (Ramakrishnan et al., 2017) . They observed that the oil obtained from matured seeds has fewer fatty acids, higher unsaturated fatty acids, more amino acids, free radical scavenging capabilities, and lower phosphomolybdenum levels. However, the steam distillation method of extraction has significant drawbacks, like extended extraction durations, high energy consumption, probable destruction of sensitive compounds due to heat, inability to separate liquids with identical boiling points, and the need for specialized equipment, which frequently requires additional processing to separate the extracted component from the water phase. As such, alternative methods are desirable.

Meanwhile, in 2021, an efficient biodiesel was reported to be synthesized using the seeds of Cassia tora (Morakinyo et al., 2021). Likewise, in 2023, a study that investigated the changes in bioactive compounds during the process of germination was reported (Islam et al., 2023).

An antioxidant is any chemical that delays, stops, or eliminates oxidative damage or cell damage to a specific molecule by neutralizing free radicals. It can be found in several foods, including fruits and vegetables. Antioxidants terminate chain reactions usually by donating electrons to free radicals, thereby preventing vital molecules and cells from being damaged (Mustafa, 2025; Sharma et al., 2025). Hence, investigation of plants with antioxidant properties is highly desirable and important.

Hence, the purpose of this study is to investigate the antioxidant activities and characteristics of Cassia tora seed oil, thereby identifying its potential with scientific backing. To the best of our knowledge, a detailed study of this kind has not been reported, as such, the result of this research will surely contribute to the existing body of knowledge while underscoring the potentials of using plant-derived medicines.

## MATERIALS AND METHODS

### Collection and Identification of the Sample

Fresh and healthy seeds of Cassia tora were picked from the plant along with their seeds cover from Arjali village area, Potiskum Local Government Area, Yobe State, Nigeria. The sample was authenticated at the Botany section of the Department of Biological Sciences of Yobe State University Damaturu, Nigeria

The matured Cassia tora seeds used in this study, shown in Figure 1, were found and collected from the Arjali village area of Potiskum, Yobe State, in northeastern Nigeria.

### Sample Preparation

The sample was dried under the shade in the laboratory at room temperature. The seeds cover was removed and then pulverised in a hardwood mortar and pestle, and it was further sieved with the Supertek standard sieve (0.5mm). The sample was weighed and transferred into a labeled container until required for analysis.

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**Figure 1:** *Cassia tora* plant and its seeds.

### Sample Extraction

Using an Ultrasonic extractor, the sample was successively extracted using three solvents in the order of increasing polarity (n-hexane, ethyl acetate, and distilled water). The sample (50 g) was weighed and put into a clean, dried, and labeled container. The container was filled up with 100ml of n-hexane and placed in an ultrasonic bath at a temperature of 60- 80 °C for 30 min. It was then filtered into a beaker using filter paper in a fume hood. The residue was washed with 50 ml of n-hexane and filtered. The three filtrates were pooled in a sealed, labeled container, and stored for solvent recovery. The procedure was repeated with the residue using the other solvent (ethyl acetate and distilled water) as described in the literature (Hasler & Blumberg, 1999)

### Solvent recovery

The extract was set up onto a rotary evaporator that was connected to a receiving flask and a condenser. It was then carefully placed within the water bath that was connected to the rotary evaporator, which was then turned on to begin the solvent distillation to about 25 ml. The concentrated extract was transferred into a pre-weighed and dried crucible in a fume cupboard until it reached a constant weight for each extract. The weight of the extract was recorded, and the extract was transferred into a container, labeled, and kept for further analysis.

### Antioxidant Activities Test

Six test tubes were utilized to prepare aliquots of five concentrations (0, 15, 30, 60, 120, and 240 µg/ml) of the extracts and blank. The samples and standard (ascorbic acid) were precisely weighed and diluted in ethanol to achieve the desired quantities using the dilution procedure. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was quantified and diluted in ethanol to produce a 0.004% (w/v) solution. A vertex mixer was employed to dissolve the material uniformly. After achieving the required concentrations, 3 ml of 0.004% DPPH was added to all test samples and the standard ascorbic acid solution. The room temperature was measured, and the test tubes were stored in a dark spot for 30 minutes to finish the reactions. At the same time, DPPH was introduced to the blank test tube (which had previously only contained ethanol). A UV-visible spectrophotometer was used to measure the absorbance of each test tube combination after 30 minutes at 517 nm. The measurements of the $IC_{50s}$ were done by plotting a graph of % inhibition vs. concentration for each sample and standard ascorbic acid by using MS Excel. This method, although a bit modified is usually what is reported in the literature

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(Christodoulou et al., 2022; Munteanu & Apetrei, 2021; Shahidi & Zhong, 2015)

**FTIR Analysis**

A KBr pellet was prepared with 0.5g of Cassia tora oil, the FTIR spectra was collected at 4000-400 $cm^{-1}$. The FTIR spectroscopic analysis of Cassia tora seed oil was carried out to determine its molecular structure and functional groups. Liquid samples are placed directly onto the sandwiched between two infrared-transparent windows, such as potassium bromide (KBr) plates (Olori et al., 2021)

**Physicochemical Properties**

The physicochemical and fatty acids content of the Cassia tora seed oil was investigated. The saponification value, iodine value, acid value, color, and oil content were all determined in this study.

***Oil content***

The powdered sample was extracted sequentially with n-hexane using an ultrasonic extractor. The results of the extraction oil content were obtained using the formula below.

$$\% \text{ Oil Content} = \frac{\text{weight of extract}}{\text{weight of sample}} \text{ X } 100 \quad \ldots\ldots\ldots\ldots.. (1)$$

***Saponification value***

Saponification value was obtained according to ASTM D5558-95. Weight 0.5g of Cassia tora oil was added to a 250ml flask, and 10ml of potassium hydroxide (KOH) solution was added. The mixture was placed in a water bath for 30 minutes, allowing the oil to react entirely with KOH until saponification was accomplished. After adding a few drops of phenolphthalein indicator, the solution was titrated with hydrochloric acid (HCL) until the pink colour faded (Sakthivel et al., 2018).

***Iodine value***

According to ASTM D5554. Cassia tora oil (0.5g) was put to a dry, clean conical flask. The oil was then thoroughly dissolved by adding 10ml of carbon tetrachloride, followed by the addition of 10ml of Wijs solution (iodine monochloride) to a flask containing the dissolved oil sample. The combination was left to react for 30 minutes in the dark. The flask was then filled with 100 ml distilled water and 20 ml of potassium iodide (KI) solution. The solution was titrated with sodium thiosulphate until it became pale yellow. The solution turned blue after adding a few drops of starch indicator solution (Sakthivel et al., 2018).

***Acid value***

A 0.5g of Cassia tora oil was carefully added to a 250 ml conical flask, along with 10 ml of neutralized ethanol (previously boiled with KOH and cooled). The oil was dissolved, and 1-2 drops of phenolphthalein indicator solution were added to the flask. The solution was titrated with potassium hydroxide solution and shaken until a pink colour developed according to ASTM D664 (Sakthivel et al., 2018).

## RESULTS AND DISCUSSION

**Physicochemical Properties**

The physicochemical parameters (given in Table 1) revealed that the seed oil of Cassia tora is dark brown in color. The acid value of the oil is 1.19 mg/g and the saponification value of the oil is 187.71mgKOH/g. Acid value is a measure of rancidity induced by chemical or enzymatic hydrolysis. An acid value of 1.19 mg/g implies that the sample has a low amount of free fatty acids, indicating good quality, minimum rancidity, and appropriate freshness for oils or fats (Gadwal & Naik, 2015).

The saponification value is the oil's ability to form soap. It is useful for calculating the

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quantity of alkali required for soap manufacture, evaluating oil quality, and identifying oil type. Saponification values of 187-190 are typical of common edible oils containing long-chain fatty acids. These types of oil are commonly non-drying or semi-drying oils. Oils with this saponification value can be used to make hard, mild soaps and are regarded for their stability and nutritional character (Ivanova et al., 2022; Khan, 2018).

The iodine value of the oil is 7.64 mg/g and is regarded as the measure of the degree of unsaturation in the oil. A 7.6 mg/g iodine value implies a highly saturated fat, oil, or wax, which has important consequences for its stability, physical qualities, and industrial applications. A lower iodine value in oils and fats indicates better saturation and less unsaturation. Specifically, it shows that the oil or fat has fewer double bonds (where iodine is added) and is predominantly composed of saturated fatty acids. This frequently results in properties such as higher melting points and improved stability (Onu & Mbohwa, 2021).

**Table 1:** Physicochemical Properties

| S/N | Parameters | Concentration (mg/g) |
|---|---|---|
| 1 | Saponification value | 187.71 |
| 2 | Iodine value | 7.64 |
| 3 | Acid value | 1.19 |
| 4 | Oil content (%) | 20.8 |
| 5 | Color | Dark brown |

**Oil Content**

Percentage Oil content of the seeds is shown in Table 2. As can be observed, the n-hexane extract contains more oil than the other extracts. This is because N-hexane extracts produce more oil than other solvents such as ethanol because of their higher affinity for oils and non-polar character, making them a better solvent for extracting non-polar molecules such as lipids (Agu et al., 2024).

**Table 2:** Percentage Oil content of n-hexane extract of Cassia tora seeds

| S/N | Solvent | Weight of sample (g) | Weight of extract (g) | % Oil content |
|---|---|---|---|---|
| | n-hexane | 50 | 10.4 | 20.8 |
| | Ethyl acetate | 50 | 2.4 | 4.8 |
| | Distilled water | 50 | 1.3 | 2.6 |

**Antioxidant Activity**

Antioxidant activity studies analyze compounds' ability to neutralize free radicals and prevent oxidative damage, frequently utilizing experimental assays to determine scavenging, reducing, or metal chelation activities (Sampath et al., 2022) . The $IC_{50}$ value in free radical scavenging represents the concentration of a chemical required to scavenge 50% of the original DPPH radicals. A lower $IC_{50}$ value often suggests more radical scavenging capacity and stronger antioxidant activity (Olugbami et al., 2014). DPPH is a stable free radical used to measure the antioxidant activity of different substances. It is a dark-colored molecule that loses its purple color when exposed to antioxidants, turning yellow. A spectrophotometer can quantify this color shift, which allows researchers to estimate a substance's antioxidant potential (Heim et al., 2002).

Figure 2 shows the $IC_{50}$ value for free radical scavenging ability of Cassia tora seed oil. The total antioxidant capacity of *Cassia tora* seed oil was determined by forming a phosphomolybdenum complex solution. The complex formation at $95^0$ was determined by

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the intensity of absorbance at (695nm) in oil at concentrations of 50-300µg/ml.

**Table 3:** DPPH Scavenging Radical Activity.

| S/N | Concentration (µg/ml) | Abs of control | Abs of sample | RSA (%) | $IC_{50}$ |
|---|---|---|---|---|---|
| 1. | 15 | 1.3 | 1.2 | 7.7 | 202.9 |
| 2. | 30 | 1.3 | 1.1 | 15.3 | 202.9 |
| 3. | 60 | 1.3 | 1.0 | 23.1 | 202.9 |
| 4. | 120 | 1.3 | 0.8 | 38.5 | 202.9 |
| 5. | 240 | 1.3 | 0.6 | 55.7 | 202.9 |

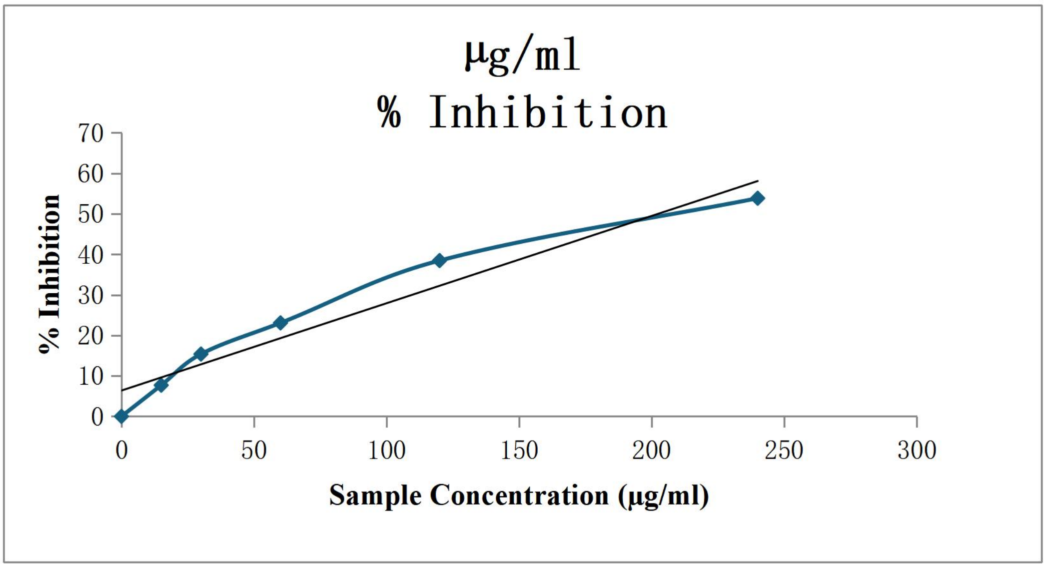


**Figure 2:** Graph of % inhibition of n-hexane extract against sample concentration (µg/ml).

As the concentration of the inhibitor decreases, the percentage of inhibition typically decreases as well. This is because inhibition is often dependent on the concentration of the inhibitor. At higher concentrations, more inhibitor molecules are available to interact with the (free radicals), leading to greater inhibition. As the concentration decreases, fewer inhibitor molecules are available, leading to a lower level of inhibition and thus a decrease in the percentage of inhibition (CORTÉS et al., 2001; Saboury, 2009). However, the relationship between sample concentration and % inhibition can vary depending on the type of inhibition and the system being studied.

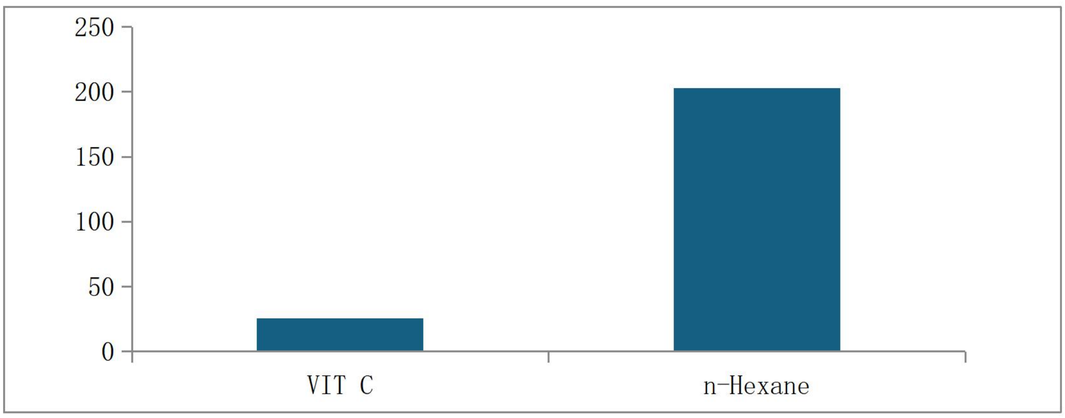


**Figure 3:** Free radical scavenging ability of the seed oil of *Cassia tora.*

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Stronger $IC_{50}$ (lower $\%IC_{50}$ value), means the standard (Vit. C) is more potent as a lower concentration is needed to inhibit the function by 50%. Weaker $IC_{50}$ (higher $\%IC_{50}$ value), indicates the n-hexane extract is less potent, as a higher concentration is needed to inhibit the function by 50%. As a result, the n-hexane extract with a higher $\%IC_{50}$ value is weaker since it requires more of the extract to accomplish the same inhibition. A lower $IC_{50}$ value suggests greater inhibition/potency, whereas a larger $IC_{50}$ value implies lesser inhibition/potency. This is reported in numerous literature (Berrington & Lall, 2012; Berrouet et al., 2020)

## FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy is an analytical technique used to detect and characterise chemical substances through determining the absorbance of infrared light by a sample's molecular bonds. The method relies on the interaction between infrared radiation and the chemical bonds in a molecule, which results in vibrational transitions that are specific to the molecular structure (Mohamed et al., 2017) . The Cassia tora seed oil was characterized by FTIR spectroscopy to determine possible bioactive compounds and potential for technological application in industries and pharmacology. The FTIR spectrum of Cassia tora seed oil provides insight into its chemical composition, with several characteristic absorption bands indicating the presence of specific functional groups. The samples' principal peaks were: ~3677 $cm^{-1}$, due to OH stretching vibration in hydroxyl groups (Trittschack & Grobéty, 2013). This signifies the presence of free hydroxyl groups typical of alcohols or phenolic compounds. Recent studies have shown that phenolic compounds in plant oils contribute significantly to their antioxidant properties, which may be relevant to Cassia tora seed oil's potential health benefits (Nkwocha et al., 2023) . The presence of hydroxyl groups suggests moisture content, while the ester groups indicate the potential for oxidation (Farhoosh et al., 2016)

The peak at 3582 $cm^{-1}$ suggests the presence of N-H stretching vibration in both primary and secondary amines (Zaharani et al., 2025) The peak at ~3300, ~3319, and ~3047 $cm^{-1}$ signifies the existence of C-H stretching vibrations in alkanes and alkyl groups (Liu, 2021) . The vibration peaks at ~ 2448 and 2588 $cm^{-1}$ are attributed to C-N stretching vibration in nitriles (Singh et al., 2015).

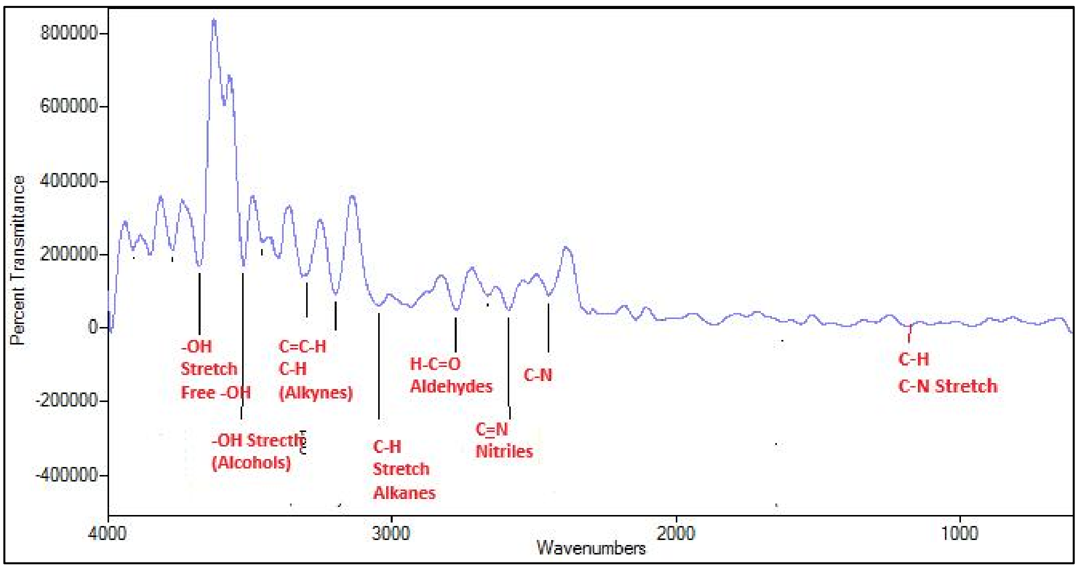


**Figure 4:** FTIR spectra of *Cassia tora* seed oil.

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It is however important to know that the FTIR results of Cassia tora leaves and stem indicated the presence of triglycerides, with a high content of unsaturated fatty acids, as such the seed will most likely have the same properties (Shukla et al., 2018) . The sharp peak near 3300 $cm^{-1}$ is associated with C≡C-H stretching, characteristic of alkynes. This indicates the potential presence of alkyne structures, which may arise from the oil's minor unsaturated components (Asemani & Rabbani, 2020) . Overall, the FTIR analysis confirms that Cassia tora seed oil contains functional groups typical of natural oils, including alcohols, esters, hydrocarbons, and nitrogenous compounds. These findings are consistent with recent studies highlighting the nutritional and medicinal value of plant oils, particularly in their fatty acid composition and bioactive components.

## CONCLUSION

Freshly picked Cassia tora seeds were extracted with n-hexane, ethyl acetate, and distilled water. The antioxidant potential of the extracted oil was studied, and scavenging activity tests were performed. The results demonstrated that the oil has an unusual antioxidant activity when compared to typical antioxidants, indicating its potential in a variety of applications and goods. The FTIR spectroscopy verified the presence of hydroxyl, esters, aliphatic hydrocarbons, carbonyls, aldehydes, and unsaturated bonds, all of which are indicative of fatty acids and triglycerides. The saponification value of 187.71mg KOH/g, iodine value of 7.64mg/g, and acid value of 1.19mg/g were determined. The findings of this study reinforced Cassia tora oil's potential in a variety of applications, as well as its ability to be employed as an antioxidant or as part of formulations containing a variety of effective antioxidants.

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