www.ijarp.com ISSN 2456-9992 Page: 01-08
International Journal Advanced Research Publications
Research Article
Volume: 02
Issue: 06

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF ETHANOLIC SEED EXTRACT OF *CASSIA TORA* IN HEPATOTOXIC RATS

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Article Received: 25 April 2026, Article Revised: 15 May 2026, Published on: 05 June 2026

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DOI: https://doi-doi.org/101555/ijarp.9877

ABSTRACT

Herbal medicines are widely used because they contain natural compounds that may help prevent and treat diseases. This study evaluated the antioxidant activity of ethanolic seed extract of *Cassia tora*in carbon tetrachloride (CCl_4)-induced hepatotoxic rats. Qualitative phytochemical screening of the extract showed the presence of flavonoids, anthraquinones, saponins, steroids, and terpenoids.Antioxidant analysis showed that treatment with the extract significantly ($p < 0.05$) increased the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) at doses of 200 mg/kg and 400 mg/kg. The level of malondialdehyde (MDA), which is an indicator of oxidative stress, was significantly reduced, especially at 400 mg/kg. Catalase (CAT) activity increased slightly, although the increase was not statistically significant.The findings suggest that ethanolic seed extract of *Cassia tora*possesses antioxidant and hepatoprotective properties. These effects may be linked to the phytochemicals present in the plant. Therefore, the extract may serve as a potential natural therapeutic agent against liver damage and oxidative stress-related disorders.

KEYWORDS: Antioxidant, *Cassia tora*, hepatotoxicity, phytochemicals, Wistar rats.

INTRODUCTION

Traditional medicine is a system of healthcare that uses natural substances and cultural practices to prevent and treat diseases (Tom *et al*., 2008). Many people in developing countries depend on traditional medicine because modern healthcare services are expensive

or not easily available (Omonike, 2010). Herbal plants are commonly used in treating many diseases because they contain important bioactive compounds (Palaniveluet *al*., 2015).

Medicinal plants have played an important role in human health for many years. Several modern drugs were originally obtained from plants. These plants contain natural compounds that may protect the body against diseases and oxidative stress.

*Cassia tora*is a plant commonly found in forests, roadsides, and fallow lands. Its seeds are rich in nutrients and are also used in traditional medicine. Previous studies have reported that *Cassia tora*possesses hepatoprotective, anti-inflammatory, antimicrobial, and antioxidant activities (Upadhyayet *al*., 2000; Gobianandet *al*., 2010).

Antioxidants help protect the body against damage caused by free radicals. Oxidative stress occurs when free radicals become excessive in the body and damage cells and tissues. Flavonoids and other phenolic compounds present in plants are known to act as antioxidants because they can neutralize free radicals (Sarianet *al*., 2017; Gismondiet *al*., 2017).

Several studies have shown that *Cassia tora*contains phytochemicals with antioxidant and medicinal properties (Dai and Mumper, 2010; Heim *et al*., 2002). Although many studies have been conducted on this plant, more investigations are needed to confirm its antioxidant potential in different regions and conditions. Therefore, this study evaluated the phytochemical constituents and antioxidant activity of ethanolic seed extract of *Cassia tora*in CCl_4-induced hepatotoxic rats.

STATEMENT OF THE PROBLEM

The liver is an important organ responsible for metabolism, detoxification, and excretion of harmful substances from the body. Exposure to toxins, drugs, alcohol, and environmental pollutants can damage the liver and lead to serious health problems (Saleem, 2008).

Although synthetic drugs are available for the treatment of liver diseases, many of them are expensive and may cause side effects. As a result, there is a growing need to explore natural and affordable medicinal plants with hepatoprotective and antioxidant properties. Therefore, this study was designed to investigate the antioxidant potential of ethanolic seed extract of *Cassia tora*in hepatotoxic rats.

AIM AND OBJECTIVES

Aim

To evaluate the antioxidant activity of ethanolic seed extract of *Cassia tora*in hepatotoxic rats.

Specific Objectives are to:

i. determine the phytochemical constituents of ethanolic seed extract of Cassia tora.

ii.evaluate the in vivo antioxidant activity of ethanolic seed extract of *Cassia tora*in CCl_4-induced hepatotoxic Wistar rats.

MATERIALS AND METHODS

Materials

Equipment

The equipment used in this study included weighing balance, crucible, measuring cylinder, round-bottom flask, test tubes, beakers, pestle and mortar, incubator, Whatman filter paper, syringes, spatula, cotton wool, micropipettes, centrifuge, spectrophotometer, serum bottles, and Soxhlet extractor.

Chemicals and Reagents

The chemicals and reagents used included distilled water, ethanol, olive oil, sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrogen peroxide, potassium phosphate buffer, nitroblue tetrazolium (NBT), sodium benzoate, carbon tetrachloride (CCl_4), thiobarbituric acid, sodium azide, and sodium EDTA.

Experimental Animals

Healthy Wistar rats were obtained from the Animal House of the Department of Biochemistry and Chemistry. The animals were kept in clean cages under proper ventilation and were fed standard animal feed and water ad libitum. The cages were cleaned regularly throughout the experiment.

Collection and Preparation of Plant Sample

The seeds of *Cassia tora*were collected from the Polytechnic environment behind the School of Science and Technology Complex. The seeds collected were washed thoroughly and air-dried under shade. The dried seeds were ground into powder using a mortar and pestle.

Ethanolic Extraction of *Cassia tora*Seeds

One hundred grams (100 g) of powdered seed sample was extracted using a Soxhlet extractor with 70% ethanol and 30% distilled water in a ratio of 1:10 (w/v) according to the method described by Azwanida (2015). The extraction process lasted for three days. The extract obtained was concentrated and dried in a porcelain dish. The percentage yield obtained was 12.53% (w/w).

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The ethanolic seed extract of *Cassia tora*was screened for the presence of major phytochemicals using standard methods.

Determination of Anthraquinones

Bontrager's test was used to determine the presence of anthraquinones. A pink or violet coloration indicated a positive result (Trease and Evans, 2002).

Determination of Flavonoids

The extract was treated with ethanol, concentrated hydrochloric acid, and magnesium. A reddish-orange color indicated the presence of flavonoids.

Determination of Alkaloids

Meyer's reagent was added to the extract filtrate. Formation of a cream precipitate indicated the presence of alkaloids.

Determination of Phenols

Ferric chloride and lead acetate tests were used to determine phenolic compounds.

Determination of Saponins

The frothing test was used. Persistent frothing indicated the presence of saponins.

Determination of Tannins

Ferric chloride solution was added to the extract. A brownish-green coloration indicated the presence of tannins.

Determination of Steroids

Acetic anhydride and concentrated sulfuric acid were added to the extract. A violet-to-green color change indicated the presence of steroids.

Determination of Terpenoids

The extract was mixed with chloroform and concentrated sulfuric acid. A reddish-brown coloration confirmed the presence of terpenoids.

EXPERIMENTAL DESIGN

A total of thirty (30) healthy Wistar rats were used for the study. The rats were acclimatized for seven days and divided into five groups of six rats each.

Group I: Normal control administered distilled water.

Group II: Hepatotoxic control administered CCl_4.

Group III: Standard control treated with silymarin and CCl_4.

Group IV: Treated with 200 mg/kg of *Cassia tora*extract and CCl_4.

Group V: Treated with 400 mg/kg of *Cassia tora*extract and CCl_4.

The treatment lasted for 14 consecutive days. CCl_4 was administered intraperitoneally one hour after the final treatment. The animals were sacrificed 24 hours later under anesthesia.

IN VIVO ANTIOXIDANT ASSAY

Blood samples were collected and centrifuged to obtain serum. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) were determined using standard laboratory methods.

Statistical Analysis

All results were expressed as Mean ± SEM. Statistical analysis was performed using ANOVA and LSD post hoc test with SPSS version 20. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Analysis

The phytochemical analysis of ethanolic seed extract of *Cassia tora* revealed the presence of flavonoids, anthraquinones, saponins, steroids, and terpenoids. However, alkaloids, phenols, and tannins were absent.These phytochemicals are known to possess antioxidant and hepatoprotective activities. Flavonoids and saponins especially play important roles in scavenging free radicals and reducing oxidative stress.

Table 1 Phytochemical Analysis of Ethanolic Seed Extract of *C. tora.*

Phytochemicals	Test	Observation	Inference
Alkaloids	Meyer's test	Blueish black	-
Flavonoids	Shinoda's test	Orange	+
Anthraquinones	Bontrager's test Combined anthraquinones	Pink Violet	+ +
Phenols	Ferric acid test Lead acetate test	Bluish black Yellow	-
Saponins	Frothing's test	Frothing	+
Tannins	$FeCl_3$ test	Brownish green	-
Steroids	Salkowski's test	Yellow	+
Terpenoids	H_2SO_4 test	Reddish brown	+

Key:

Present +, Absent -

Antioxidant Activity

The results showed that administration of CCl_4 significantly reduced antioxidant enzyme activities and increased oxidative stress in rats. Treatment with ethanolic seed extract of *Cassia tora*improved antioxidant enzyme levels.

Table 2. Effect of Ethanolic Seed Extract of *Cassia tora* on Antioxidant Parameters in Wistar Rats.

EXPERIMENTAL GROUPS	SOD (µ/ml)	MDA (umol/mg)	CAT (U/mg)	GPx(µ/mg)
DH_2O (normal control)	35.14± 0.87^c	25.04± 1.46^b	15.00± 0.64^a	59.44± 0.49^a
CCl_4 (hepatotoxic control)	31.39±0.78^a	37.44± 1.59^a	13.88± 0.80^a	54.24±3.66^a
Silymarin (STD control) + CCl_4	39.09± 0.43^b	29.92± 0.81^b	17.78± 0.42^b	83.00±1.88^b
200 mg/kg extract + CCl_4	35.76± 1.75^c	35.84± 1.54^a	14.68± 0.81^a	112.54± 1.58^c
400 mg/kg extract + CCl_4	38.14± 0.83^b	31.70± 0.54^b	15.26±0.90^a	118.10± 3.10^c

Results are expressed as Mean±SEM (n=6 for each group)

Values with different superscript down the group are statistically ($p<0.05$) significant.

SOD= Superoxide Dismutase

MDA= Malonyldialdehyde

CAT= Catalase

GPx= Glutathione Peroxidase

The extract significantly increased SOD and GPx activities at both 200 mg/kg and 400 mg/kg doses. The higher dose produced better antioxidant effects. MDA levels were significantly reduced in treated groups, indicating reduced lipid peroxidation and oxidative damage.Although CAT activity increased slightly after treatment, the increase was not statistically significant.

The protective effect of the extract may be due to the presence of bioactive phytochemicals such as flavonoids, saponins, and terpenoids. These compounds are known to neutralize free radicals and protect liver cells from oxidative damage.

The findings of this study agree with previous reports that *Cassia tora*possesses antioxidant and hepatoprotective properties (Saravanan and Malarvannan, 2016; Prabhu and Krishnamoorthy, 2011).

CONCLUSION

The ethanolic seed extract of *Cassia tora* demonstrated significant antioxidant and hepatoprotective activities in CCl_4-induced hepatotoxic rats. The extract improved antioxidant enzyme activities and reduced oxidative stress markers.These beneficial effects may be attributed to the presence of phytochemicals such as flavonoids, saponins, steroids,

and terpenoids. Therefore, *Cassia tora*may serve as a potential natural source of antioxidant compounds for the management of liver disorders.

RECOMMENDATIONS

1. Standardization of the extract should be encouraged for possible pharmaceutical development.
2. Further studies should be conducted to isolate and characterize the active compounds responsible for the antioxidant activity of *Cassia tora*.
3. Clinical studies should be considered to evaluate the effectiveness of *Cassia tora* in humans.

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