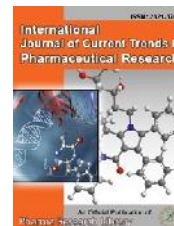




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Research Article

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Phytochemical Screening and Acute Toxicity Study of Methanol extract of "Cassia tora" Seeds

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ABSTRACT

Many plant species have been used by most ethnic groups for treating different disease conditions ranging from small infections to skin diseases, asthma, dysentery, malaria and other multiple indications. Herbal Medicine is usage of herbs for therapeutic and medicinal importance. An herb is a variety of chemical substances that act upon the body. Medicinal plants have been identified and utilized all over human history. Plants have the capacity to synthesize a plethora of chemical compounds that are used to normalize altered important biological functions, and to protect against predators such as fungi, insects, and herbivorous mammals. So we screened active phytochemical constituents and evaluated acute toxicity potential of methanol extract of seeds of *Cassia tora*. The methanol extract of seeds of *Cassia tora* showed presence of various phytoconstituents like Alkaloids, glycosides, saponins, flavonoids, fixed oils & fats, Proteins, Phenol compounds and tannins and extract was safe up to 2000 mg/kg orally proved through acute toxicity study.

Keywords: *Cassia tora*, Acute Toxicity, Phytochemical screening, Medicinal Plants.

ARTICLE INFO

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1. Introduction

The World Health Organization (WHO) estimates that approximately 4 billion people, around 80 percent of the world population, now using herbal medicines for a few

primary health care needs. Herbal medicine is playing a major contribution in all indigenous and domestic peoples' traditional medicine. Herbal medicine is a most common

ingredient in Ayurvedic, Native American Indian Medicine, Homeopathic and Naturopathic traditional oriental. WHO estimates out of 119 plant-derived pharmaceutical medicines, approximately 74 percent are used in current modern medicine used are as that of traditionally used plant medicines by native cultures. Many pharmaceutical companies are conducting major research on plant and plant derived materials collected from the forests and more other places for their potential therapeutic value¹⁻⁴.

From literature survey it was found that *Cassia tora* effective in treatment of anti-ulcer, anti-shigellosis, anti-genotoxic, anti-proliferative, anti-oxidant, anti-arthritis, wound healing activity etc due to phytochemical constituents present in it⁵⁻⁴⁰. From previous studies done on *Cassia tora*. Phytochemical screening was done to identify the active phytochemical constituents present in methanol extract of seeds and acute toxicity study was carried out determine the effective and safe dose.

2. Materials and Methods

Plant Collection

The plant seeds were collected during the month of March 2017 from Tirumala hills. The plant was authenticated by Dr. K.Madhava chetty.

Plant Extraction

Collected seeds were shade dried; when they are completely dried they were subjected for size reduction. The dried seeds are grinded to fine powder with the help of mixer. The powder is then packed in air tight plastic cover and subjected for extraction of drug with the help of soxhlet extractor.

Soxhlet Extraction⁴¹

The dried material is powdered and made ready for Extraction. The *Cassia tora* plant powder seeds are kept for reflux process. About 150 gm of plant powder was taken in a soxhlet thimble and fitted with a reflux condenser. The process was done for about 6 hrs after soaking for 2 days in 90% Methanol. The extract was taken and filtered and then it is kept for condensation to separate the solvent. After complete extraction, the extract was filtered and solvent was distilled off. The extract was concentrated to dry residue. The percentage yield of the extract was calculated with reference to air dried powder.

Preliminary Phytochemical Studies⁴²⁻⁴⁵

Phytochemical analysis for major phytoconstituents of the methanol extract from CT was undertaken using standard qualitative methods as described by various authors (Vogel, 1958; Kapoor et al., 1969; Rizk and Bashir, 1980; Fadeyi et al., 1989; Odebiyi and Sofowora, 1990). The plant extract was screened for the presence of biologically active compounds like Alkaloids, Carbohydrates, Glycosides, Flavonoids, Saponins, Phenolic compounds, Tannins, Steroids, Proteins and Amino acids and Terpenoids.

Tests for alkaloids:

A small portion of the methanol extract was continuously stirred separately with few drops of dil. HCl and filtered. The filtrate was treated with various reagents as shown for the presence of alkaloids.

Mayer's reagent - Creamy precipitate

Dragandroff's reagent - Orange brown precipitate

Hager's reagent - Yellow precipitate

Wagner's reagent - Reddish brown precipitate

Tests for carbohydrates:

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

Molisch's Test

Filtrate was treated with 2-3 drops of 1% alcoholic - naphthol solution and 2 ml of con. H₂SO₄ was added along the sides of the test tube. Appearance of violet coloured ring at the junction of two liquids shows the presence of carbohydrates.

Benedict's test

Filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugars.

Tests for glycosides:

Legal's Test: To the hydrolysate, 1ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide solution. After that pink to red color appears indicating the presence of glycosides.

Borntrager's Test:

Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink colour showing the presence of glycosides.

Tests for saponins:

20 ml of distilled water was added to the extract and it was mixed in a graduated cylinder about 15 minutes. The appearance of 1cm layer of foam shows the presence of saponins.

Tests for phenolic compounds:

Minute quantity of the extract was taken in water and investigated for the presence of phenolic compounds and tannins using following reagents.

A. Dil.FeCl₃ solution (5%)- violet color

Tests for Tannins:

1% solution of gelatin containing 10% NaCl - white precipitate 10% lead acetate solution - white precipitate.

Tests for proteins and free amino acids:

Small quantity of the extract was dissolved in few ml of water and treated with following reagents. Millon's reagent - Appearance of red colour shows the presence of protein and free amino acids.

Ninhydrin reagent:

Appearance of purple color shows the presence of proteins and free amino acids.

Biuret test:

Equal volumes of 5% NaOH solution and 1% copper sulphate solution were added. Endpoint is pink or purple colour indicating the presence of proteins and free amino acids.

Tests for flavonoids:

With aqueous Sodium hydroxide solution: Blue to violet colour indicates anthocyanins, yellow color (flavones) and yellow to orange (flavonones).

With Con. H₂SO₄:

Yellow orange colour (anthocyanins), yellow to orange colour (flavones), orange to crimson (flavonones)

Shinoda's test:

Minute quantity of the extract was added in alcohol and to that a pinch of magnesium followed by Con. HCl drop wise was added and heated. Appearance of magenta color shows the presence of flavonoids.

Animals used:

Healthy male BALB/c mice (20-22 g) were procured and acclimatized for a week to laboratory conditions before study. Mice were given food and water ad libitum. For each dose 3 animals were used.

Acute Toxicity Studies

Animals were fasted prior to dosing, food but not withheld overnight. Following the period of fasting, the animals were weighed and test substance was administered. After the substance as administered, food was withheld for further 3 to 4 hrs. After a period, animals were provided with food and water depending on the length of the period. Three animals were used for each step. The dose levels of the extract to be used as the starting dose was selected from one of the four fixed dose levels 500, 1000, 1500, 2000 mg/kg body weight. The starting dose level selected was such that which was most likely to produce mortality in some of the dosed animals. After administration of the test sample, the animals were observed continuously for first four hours behavioral changes and at the end of 48 hrs for mortality⁴⁶, if any.

3. Results and Discussions

Percentage Yield:

The formula for calculation of percentage yield is as follows

% yield= (The extract obtained / the amount of powder packed) x100

The percentage yield of methanol extract of seeds of *Cassia tora* was found to be 18.79%.

Preliminary Phytochemical Screening

Phytochemical screening of Methanol extract of *Cassia tora* seeds was done, the extract showed the presence of Alkaloids, glycosides, saponins, flavonoids, fixed oils, fats, Proteins, Phenol compounds and tannins.

Acute Toxicity Studies

The acute toxicity study of methanol extract of *Cassia tora* seeds was carried out as per OECD guidelines 423. There was no gross evidence of any abnormalities observed up to a period of 4-6 hrs and no mortality was observed at the maximum tolerated dose (MTD) level of 2000 mg/kg body weight per oral. The maximum tested dose was 2000mg/kg body weight. Further pharmacological screenings can be carried out with two dose ranges i.e. 1/8 of MTD (250 mg/kg bow p.o.), 1/4 of MTD (500 mg/kg bow p.o.). They may taken as Test doses T1 and T2 respectively.

Discussion: The secondary metabolites show significantly the biological activities of medicinal plants like antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, hypoglycemic, antidiabetic, antioxidant, antileprosy activities etc⁴⁷. Extract showed the presence of Alkaloids, glycosides, saponins, flavonoids, fixed oils, fats, Proteins, Phenol compounds and tannins. Flavonoids, the major and diverse and widespread frequent group of polyphenolic compounds that showed a wide variety of chemical and biological activities inclusive of radical scavenging properties that are found enormously in plants for normal growth development and defense against injuries and infections⁴⁸. The Acute Toxicity studies (OECD 423 Guideline) revealed that 90% Methanolic aqueous extract of seeds of *Cassia tora* was found to be practically non toxic up to a dose of 2000 mg/kg b.w when given orally which was the safe dose. The study may be conducted using two doses i.e., 250 mg/kg b.w p.o and 500 mg/kg b.w p.o.

Table 1: Percentage Yield of Methanol Extract of Seeds of *Cassia tora*

S. No.	Solvent	Color and Consistency	Percentage yield
1	Methanol 90% Seeds	Dark brown sticky	18.79%

Table 2: Data Showing Preliminary Phytochemical Screening of the Methanol Extract of seeds of *Cassia tora*

Phytoconstituents	Present or
Carbohydrates	+
Glycosides	+
Fixed oils and fats	+
Potein & amino acids	+
Saponins	+
Tannins	+
Phenolic compounds	+
Flavonoids	+
Alkaloids	+

(+) Present

(-) Absent

Table 3: Acute Toxicity observations of the Methanol Extract of Seeds of *Cassia tora*

Alertness	Behavioral Response	
Stereotypy		-
Irritability		
Fearfulness		
Touch responds		
Analgesia		N
Spontaneous activity		
Grooming		
Restfulness		
Inclined plane test		
Body Temperature		
Righting responds	Neurological Response	-
Limb tone		N
Grip strength		+
twitching		-
Abdominal tone		+
Pinnal reflex		N
Corneal reflex		N
Straub tail		+
Tremors		-
Convulsions		-
Catalepsy		-
Writhing	Autonomic Response	+
Defecation		
Urination		
Piloerection		+
SMA		N
Respiration		
Pupil size		N
Cyanosis		N
Heart rate		N
Ataxia		+
Ptosis		-
Salivation	-	
Lachrymation	-	

() Increased () Decreased (+) Presence (-) Absence (N) None

4. Conclusion

Cassia tora seeds extraction was carried out using 90% Methanolic aqueous extract. The % yield of extract obtained was 18.7%. Then the extract was subjected to Preliminary Phytochemical screening and it indicated the presence of Carbohydrates, Flavonoids, Anthraquinone

Glycosides, Tannins, proteins, Terpenes and Volatile Oils. 90% Methanolic aqueous extract of seeds of *Cassia tora* was found to be practically non toxic up to a dose of 2000 mg/kg b.w when given orally. In future, methanol extract of seeds of *Cassia tora* may be evaluated for various pharmacological activities.

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