



CASIA TORA: A PHYTOCHEMICAL SCREENING AND IN VITRO ANTHELMINTIC ACTIVITY OF IT'S AERIAL PARTS (LEAVES)

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ABSTRACT

Most of the traditional medicines are the most powerful source of biological activity. Ethnopharmacology is not just a science of the past using an out molded approach. It still constitutes a scientific backbone in the development of active therapeutics based upon traditional medicine. Natural products & especially those derived from higher plants have historically played a pivotal role in the discovery of new pharmaceuticals. **Aim:** The aim of the study is to investigate phytochemical screening of Petroleum ether and aqueous extract of *Cassia tora* and the presence of different secondary metabolites responsible for the therapeutic values of the drug like presence of Alkaloids, Glycosides, Carbohydrate, Tannins, Phenolic compounds, Proteins & Amino acids, Gums & mucilage, flavours & flavonoids,

saponins and steroids & sterols etc and also to find out the anthelmintic activity study by *in-vitro* test species *Pheretima posthuma* responded towards our plant extracts by showing the sign of paralysis and death finally **Result:** The Petroleum ether and aqueous extract yield was 10.93% w/w and 5.43% w/w. It showed the presence of alkaloids, glycosides, saponins, anthraquinone, terpenoids, phlobatanin like compounds but do not shows the presence of carbohydrate in Petroleum ether, phenolic, flavonoid and lignin. **Conclusion:** It showed the presence of alkaloids, glycosides, amino acids, saponins, Carbohydrate, anthraquinone, protein and amino acids like compounds. And the data revealed that the aqueous extract of *Cassia tora* (leaves) showed more potent anthelmintic activity as compared to petroleum ether extract.

KEYWORD: *Cassia tora*, Phytochemical, Albendazole, Anthelmintic Activity, *Pheretima posthuma*.

INTRODUCTION

Medicinal herbs contain many substances which are known to our modern and ancient civilizations for their healing properties. These medicinal plants and herbs are the sole source of active principles which are capable of curing man's ailments. These active principles differ from plant to plant due to their biodiversity. Medicinal^[1] plants are the local heritage with the global importance. World is endowed with a rich wealth of medicinal plants. So one of the medicinal plant *Cassia tora* is also a very important medicinal plant for the mankind which shows many pharmacologically as well as therapeutically effective for the different purposes. The leaves and seeds of the plant *Cassia tora* are acrid, antipatriotic, alterative, aperients, laxative, anthelmintic, dandruff, constipation, cough, hepatitis, fever and hemorrhoids ophthalmic, liver tonic, cardio tonic^[2], expectorant and also given to children having intestinal disorders. The leaves and seeds are also useful in leprosy, ringworm, flatulence, colic, dyspepsia^[1] constipation, impetigo, cough, ulcers, psoriasis, eye diseases, skin diseases, bronchitis, and cardiac disorders.^[1] The flowers of *Cassia tora* is used internally for bronchitis and asthma. Pods are used in dysentery and in eye diseases.^[1] The known Chemical components of *Cassia tora* are anthroquinones, chrysophanol, Emodin, obtusifolin, obtusin, chryso-obtusin, auranto obtuse and their glycosides.

Morphology

It is an annual herb, having 30–90 cm in height (Figure-1) The leaves are green in colour and its size is of 2.0-5.0cm, shape is lanceolate, margin is entire, venation is pinnate veins, anastomosing towards margin, apex is acute and its base is asymmetrical in nature, pinnate up to 6-8 cm long, leaflets are in 3 pairs distinctly petioled, opposite, conical at one end, ovate, oblong and base oblique. Flowers are pale yellow in color and usually nearly sessile pairs in the axils of the leaves with five petals upper one are very crowded. Pods are subteret, 6-12 inch long, and having numerous brown oblong, rhombohedra seeds. The Colour of seeds are Light Brown and its size is 0.3-0.4 cm long, Taste is having bitter salty and slightly cold in nature. Its odour is odourless or sometimes slightly bitter odour.



Figure-1 Plant of *Cassia tora*.

Taxonomical classification

Botanical name: *Cassia tora* Linn,

Family :Caesapiniaceae

Kingdom :Plantae

Division :Magnoliophyta

Class :Magnoliopsida

Sub-class :Rosidae

Order :Fabales

Family :Fabaceae

Sub-family :Caesalpinioideae

Tribe :Cassieae

Sub-tribe :Cassiinae

Genus :Cassia

Species :Cassia tora

Common (Indian) names

Hindi : Charota, Chakvad, Chakavat.

Bengali : Chakunda

Oriya : Chakunda

Gujrati : Kawaria

Canarese : Gandutogache

Malyalam :Chakramandrakam, takara

Marathi :Takala

Sanskrit :Chakramarda, Dadmari, Dadrughra, Taga

Tamil :Tagarai

Telugu :Chinnakasinda

PHYTOCHEMISTRY

Leaves

Preliminary phytochemical screening of leaf shows that it contains polyphenols, emodin, kaempferol-2-diglucoside, chrysophanol, aloe-emodin rhein, glucose, sennoside, stachydine, amino acids, fatty acids, ononitol monohydrate, mannitol, β -sitosterol, myricyl alcohol, Kaempferol 3-diglucoside, trigonelline, choline Sennosides. All these are well known for their medicinal properties.^[2]

Seed

Seeds contain anthraquinones as aurantio-obtusin, naphthopyroneglucosides, cassiaside, rubrofusarin-6-O- β -D gentiobioside, obtusifolin-2- β -D-glucoside, chrysoobtusin-2-O- β -D-glucoside, physcion, chrysoobtusin, emodin, chrysophanol, obtusifolin, Seeds contain castasterone, Brassinosteroids Brassinolide, typhasterol, teasterone, and 28- norcastasterone, Monoglycerides as monopalmitin and monoolein.^[2] It also contains phenolic glycosides such as rubrofusarin triglucoside, nor rubrofusarin gentiobioside, demethylflavasperone gentiobioside, torachryson gentiobioside, torachryson tetraglucoside. Seeds also contain Rhein, questin, 2-hydroxyemodin 1-methylether, Aloe emodin, Rubrofusarin and its 6- β gentiobioside, Norrubrofusarin, 8-hydroxy-3- methylanthraquinone-1 β gentiobioside, Chrysophanic acid & its 9-anthrone, Aurantio-obtusin, 1-desmethyaurantio obtusin, 1-desmethylchryso-obtusin, torlactone, torachryson, Sitosterol. Seeds also contain Rubrofusarin, triglucoside, isorubrofusarin, alaternin and adenosine, Quercetin, 6-O- β -D glucoside, 6-O- β Dgentiobioside.^[3]

Stem bark

The stem bark contain anthraquinone like, 1-hydroxy-5-methoxy-2-methyl anthraquinone, 5-methoxy-2- methyl anthraquinone-1-O- α -L-rhamnoside along with chrysophanol, emodin and β -sitosterol, d-mannitol, myricyl alcohol, β -sitosterol, glucose, tigonelline, 1- stachydine and choline^[2] and polyphenolic anthraquinone.^[4]

Root

Root contain Choline, 1,3,5-trihydroxy-6,7-dimethoxy-2 methyl anthraquinone, myricyl alcohol, chrysophanic acid, naphtho- α -pyrone, physcion, rubrofusarin & its 6 β - gentiobioside, torlactone, leucopelargonidin-3-O- α - L-rhamnopyranoside, β sitosterol.^[3] Chrysophenol is its marker compound of this plant.

Uses

Traditionally the fresh leaves of *Cassia tora* plant is used in the treatment of ulcers, piles, hemorrhoids, ring worm and other parasitic skin diseases like ring worm, rashes, allergies psoriasis, eczema and dermatomycosis. The seeds of *Cassia tora* are great laxatives, ophthalmics, anthelmintics and expectorants. The seeds of *Cassia Tora* are used as excellent sources for cooling down the body. It is used as aperients and purgatives and the seeds are help to loosen the bowels to relieve constipation. It is also used for treating childhood teething, fever and constipation. The seeds are also used as a mordant in dyeing. It can be used as a full of protein feed for livestock and birds. The fruits of *Cassia tora* are used in the treatment of fever. It is also used as a nerve tonic, heart tonic. It's root is used in the treatment of snakebite. The herb helps the body in maintaining the normal level of cholesterol. They can be roasted and ground to be used as a coffee substitute. Its powder proves useful in combating indigestion, toning up heart muscles and purifying blood. It is also used as an antidote in case of various poisonings. The leaves and seeds of *Cassia tora* are useful in leprosy, flatulence, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders. Pharmacologically this plant shows much activity as anti-bacterial activity, fungicidal activity, anti-viral activity. anti-rheumatic activity, anti-asthenic, diuretic activity, and anthelminthiasis.

MATERIALS AND METHODS

The different Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent, Wagner's, Dragendorff's, Fehling's A & B, α -naphthol, Ferric chloride, Conc. Sulphuric acid, Pyridine, Sodium nitropruside, Acetic anhydride, were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform, and Ethanol were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer. Other chemicals were prepared in the laboratory as, 10% Lead acetate, 10% Ammonium hydroxide solution, 10% Ammonia. And to carry out the anthelmintic study of different extracts, we have taken following chemicals as Saline water (Claris Lifesciences Ltd., Ahmedabad). Albendazole (Alkem Ltd.) is used as reference standards.

EXPERIMENTAL WORK

Plants collection, Identification and processing

The plant (Figure-1) *Cassia tora* was collected from adjoining area of Barpali (Dist-Bargarh, Odissa) in the month of July-2018. The plant was identified by Botanist Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D, P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur, Ganjam, Odisha. The plant was washed properly with water to remove the mud or dust if any; initially it was dried in sunlight for an hour and shade dried completely. Also all the foreign matters like dead or destructed part were removed precautionary. The plant *Cassia tora* was cut into small pieces by means of wood grinder and knives.

Preparation of the extracts

- (a) Petroleum ether Extract
- (b) Aqueous Extract

(a) Petroleum ether extract

The shade dried course powder of *Cassia tora* (200 gm) was packed well in soxhlet apparatus and was subjected for continuous hot extraction with Petroleum ether extract for 8 hours at 50°C for five days. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely and to obtain a semisolid product. The dried product was kept in the desiccators till experimentation. Then the extract was weighed and it's percentage yield was calculated in terms of air-dried powdered crude material.

b) Aqueous extract

The shade dried course powder of *Cassia tora* (200 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with distilled water for 8 hours at 50°C for five days. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely and to obtain a semisolid product. The dried product was kept in the desiccators till experimentation with the use of anti-microbial agents as it has the presence of water which has the chance of microbial contamination. The obtained extract was weighed and percentage yield was calculated in terms of air-dried powdered crude material. The yield and % yield of both Petroleum ether and aqueous extracts of *Cassia tora* was reported.

Table 1: % Yield values of Petroleum ether and aqueous extracts of *Cassia tora*.

Sl. No.	Extracts	% Yield (w/w)	Colour of extract
1.	Petroleum ether	10.93%	Greenish Black
2.	Aqueous	5.43%	Dirty Green

QUALITATIVE PHYTOCHEMICAL ANALYSIS^[5-9]

Both Petroleum ether and aqueous extracts obtained by the powdered rhizome of *Cassia tora* was subjected to various qualitative tests for the identification of different phytoconstituents present in it. The constituents present in different extracts of *Cassia tora* are summarized.

Table No 2: Phytochemical analysis of Petroleum ether & aqueous extracts of *Cassia tora*.

Phytochemical test	Petroleum ether Extract	Aqueous Extract
Alkaloid test		
Mayer's test	+ve	+ve
Wagner's test	+ve	+ve
Hager's test	+ve	+ve
Dragendorff's test	+ve	+ve
Carbohydrates		
Molish's test	-ve	-ve
Fehling's test	-ve	+ve
Benidict's test	-ve	+ve
Borntrager's test	-ve	+ve
Saponins		
Foam test	+ve	+ve
Proteins & amino acid		
Xanthoprotic test	+ve	+ve
Biuret's test	-ve	-ve
Ninhydrin test	-ve	+ve
Phenolic compounds & flavonoids		
Ferric chloride test	-ve	-ve
Lead acetate test	-ve	-ve
Alkaline test	-ve	-ve
Phytosterol :		
Liebermann-Burchard's test	-ve	+ve
Glycosides		
Modified Bront.	+ve	+ve
Cardiac Glucoside	+ve	+ve
Terpinoids		
Salkowski test	-ve	+ve
Cu. Acet. test	+ve	+ve
Lieberman test	-ve	+ve
Tannin		
Alkaline reagent	-ve	+ve
Lignin		

Phloroglucinol	-ve	-ve
Toluene	-ve	-ve
Phlobatannin Anthraquinone		
Nitric acid	+ve	+ve
Benzene	-ve	+ve

Present (+): Absent (-):

Table No 3: Powder analyses with chemical reagent.

Reagents	Colour of powder
Powder as such	Dark Green
Powder + conc. HCl	Greenish Black
Powder + conc. HNO ₃	Reddish Brown
Powder + conc. H ₂ SO ₄	Greenish Black
Powder + glacial acetic acid	Yellowish Brown
Powder + dil. HCl	Dark Green
Powder + NaOH sol.	Greenish Black
Powder + FeCl ₃	No change
Powder + picric acid	No change
Powder + ammonia	Greenish Black
Powder +Iodine	Brown

Table No 4: Fluorescence analysis of powder drug.

Reagent	Ultra Short	Ultra Long	Visible
Powder as such	Dark Green	Dark Green	Dark Green
Powder + 1N NaOH in methanol	Dark Green	Black	Dark Green
Powder + NaOH in water	Black	Black	Dark Green
Powder + HCl	Black	Black	Dark Green
Powder +H ₂ SO ₄	Black	Black	Dark Green
Powder +HNO ₃	Black	Greenish Black	Reddish Brown
Powder + pet.ether	Green	Dark Green	Black
Powder + chloroform	Brown	Light Green	Black
Powder + Dil HNO ₃	Black	Black	Reddish Brown
Powder + FeCl ₃	Brown	Light Green	Black
Powder + methanol	Dark Brown	Dark Green	Black

DETERMINATION OF ANTHELMINTIC ACTIVITY^[10]

The anthelmintic study was done by using one in-vitro species adult earthworms *Pheretima posthuma*. Earthworms were collected near the swampy water in our locality. The average size of the round worm was 5-7 cm; average size of the earthworm was 8-9 cm. These earthworms were identified and services of veterinary practioner were utilized to confirm the identity of worms. The suspensions of various extracts were prepared in 2% gum acacia solution to obtain 1, 2.5 and 5% concentrations. Solutions of similar concentrations of the standard drug albendazole were also prepared in distilled water.

Two ml of each concentration of various extracts of *Cassia tora* and standard drug albendazole were diluted to 10 ml separately with normal saline and poured in petridishes. 2ml of 2% gum acacia solution was diluted to 10ml with normal saline to serve as control. Six earthworms of nearly equal size were placed in each Petridis at room temperature. Time was recorded at the time of releasing the earthworms to each concentration. The time taken (minutes) for the complete paralysis and death were recorded. The mean paralysis time for each sample was recorded. The anthelmintic activity was evaluated on adult Indian earthworm *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colour.

Table 5: Anthelmintic effect of *Cassia tora* extracts.

Group	Concentration of Extract (%)	Time in minutes (Mean \pm SEM)	
		Paralysis time(Min)	Death time(Min)
Albendazole (standard)	10 mg/ml	14min,18 sec \pm 17	18min,18 sec \pm 42
	30 mg/ml	10min,21 sec \pm 12	14 min,12 sec \pm 10
	50 mg/ml	8 min,12 sec \pm 14	12min,40 sec \pm 11
Petroleum ether extract	15 mg/ml	27min,16 sec \pm 17	32min,15 sec \pm 48
	30 mg/ml	23min,26 sec \pm 12	30 min,26 sec \pm 12
	50 mg/ml	21 min,48 sec \pm 14	24 min,48 sec \pm 14
Aqueous extract	15 mg/ml	12min,19 sec \pm 17	20min,15 sec \pm 48
	30 mg/ml	12min,26 sec \pm 12	16 min,26 sec \pm 12
	50 mg/ml	10 min,48 sec \pm 14	13min,14 sec \pm 10
Control	-	-	-

Results are expressed as mean \pm SEM from six observations, *Control worms were alive upto 24 hrs. of observation*, N/A= No Activity shown within 24 hours.

RESULTS AND DISCUSSION

The percentage yield of Petroleum ether and aqueous extract were found to 10.93%w/w and 5.43%w/w (Table-1). The preliminary phytochemical screening on the leaf extract was carried out by subjecting the different extracts to qualitative test for the identification of various plant constituents. It showed the presence of alkaloids, glycosides, saponins, anthraquinone, terpenoids, phlobatanin like compounds (Table-2) but do not shows the presence of carbohydrate in Petroleum ether, phenolic, flavonoid and lignin. The Powder analysis and fluorescence was observed in ultra short, ultra long and visible. The results were shown in (table-3 and 4). The results (Table-5) depict the time taken for paralysis and death

of earthworms after the treatment with the test extracts at the selected concentrations. The data revealed that the aqueous extract has a better wormicidal effect than Petroleum ether extract with compared with the standard drug Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

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