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PHYTOCHEMICAL SCREENING AND ANTIHELMINTHIC ACTIVITY OF LEAF AND ROOT EXTRACTS OF CASSIA TORA PLANT

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ABSTRACT
Cassia tora is one of the most important sources of medicinally important phytochemicals and widely used in Ayurvedic and Chinese system of medicine. The fresh plants of *cassia tora* were collected from the different locality of Dharan, Sunsari district during the month of August. In this study leaves and root extracts were subjected to extraction by soxheletion by using ethanol and water and the extracts were subjected to antibacterial activity against *Staphylococcus aureus.* and *Citrobacter koseri,* the ethanolic extracts were screened for antihelmenthic activity against Indian adult earth worm (*Pheretima posthuma*) with a moderate result. The result of antibacterial activity revealed that aqueous extract of leaves and roots showed better activity in comparison to aqueous extracts particularly against gram positive bacteria (*Staphylococcus aureus)*.

INTRODUCTION

Medicinal plants are of great value in the field of treatment and cure of diseases. Over the years, scientific research has expanded our knowledge of the chemical effects and composition of the active constituents which determine the medicinal properties of plants [1]. Cassia (*caesalpiniaceae*) is one of the largest genera with about 2500 species widely distributed through the tropical and subtropical regions of the world. This genus is prized for its agricultural, economical, and medicinal virtues. Hence *Cassia* has drawn the attention worldwide. One such important medicinal plant species which belongs to this genus is cassia *tora* [2]. *Cassia tora* is one of the most important sources of medicinally important phytochemicals and widely used in Ayurvedic and Chinese system of medicine.

Cassia tora is medicinal plant but it is known to us as weed because of lack of advance technology in our country to know the active chemical constituents of the easily available plant as

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their agricultural benefits to the medicinal point of view. It is widely distributed in tropical and subtropical areas as weed and it was traditionally developed as ring worm plant because of its active property against fungal infections. It is the most valuable medicinal as well as nutritional shrub found as weed throughout tropical and subtropical region of Nepal. *Cassia tora* is an edible wild plant having remarkable nutritional as well as therapeutic property. This plant of seeds are roasted and dried then are used as substitute of coffee in many developing countries [3].

Most of the works have been reported on the seeds and their main pharmacological actions are based on the anthraquinone glycosides derivatives. Molecular mechanism of emodin action shows transition from laxative ingredient to an anti-tumor agent and mutagenic and genotoxic effects[4].

It is a well-known Ayurvedic medicinal plant as a laxative, antiperiodic and is useful for leprosy, ringworm, bronchitis and cardiac disorders, ophthalmic, skin diseases, cough, hepatic disorder, liver tonic, hemorrhoids. It was reported that seeds of CT has antioxidant activity and contain many active substances including chrysophanol, emodin, and rhein etc [5].

Here in this study we focused on the antibacterial study of ethanolic and aqueous extracts of leaves and root against specific bacterial strains like *Citrobacter koseri* (as very few or no related work was conducted on this very strains earlier) along with *S. aureus* and to compare the individual potency against these two strains. Side by side their antihelmenthic activity was also performed for aqueous extracts against Indian earthworms taking praziquantel as standard drug.

MATERIALS & METHODS

Plant collection and authentication

The fresh plants of *Cassia tora* were collected from the different locality of Dharan, Sunsari district of Nepal during the month of August.

Worm collection and authentication

The adult earthworms *Pheritima posthuma* were collected from pond area of Tarahara Sunsari district, and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The worm was authenticated by the Central College of Food Technology Hattisar, Dharan, Sunsari district Nepal.

Pharmacognostical analysis

The pharmacognostical study of *Cassia tora* was performed by naked eye to detect the specific features of the plant, which helps in easy identification and avoidance of adulteration and contamination due to misidentification of plant species.

Macroscopical Analysis

Macroscopical examination was carried out with the naked eye, which gave details concerning the plant aspect, general appearance, color and odor.

Thin layer chromatography

Thin layer chromatography of different extracts was carried out by preparing glass plates using Silica gel slurry. The plates were then dried in hot air oven and a drop of each extract of leaves, and roots were placed in different plates and the plates were kept in beaker containing appropriate solvents, followed by calculation of R_f value.

Extraction

The fresh plant part was collected from locality of Dharan Sunsari district and authenticated by head of department of botany post graduate campus Biratnagar Morang district. Then all the collected plant parts were washed with clean water and cut into small pieces and were kept in solar dryer for few days and dried plant parts were crushed into powder by electric blender (electric grinder) and coarse powder was passed through sieve no 30 and sieved powder was then subjected for extraction by using Solvents (aqueous and ethanol) in the Soxhlet apparatus. The extracts were dried by rotator evaporator and stored at 4 degree centigrade for further use.

Phytochemical Screening

The phytochemical screening was done to identify the main groups of chemical constituents present in different extracts of *Cassia tora* by their color reactions with different reagents. Each extract was subjected for glycosides (anthraquinone glycoside and C-glycoside), alkaloids, terpenoids, flavonoids, reducing sugars, tannins and saponins using test procedures.

Selection of standard antibiotics

Microorganisms were obtained from Microbiology Laboratory of Sunsari Technical College. Streptomycin was purchased from local market in Dharan (Cipla, Mumbai).The purity of the antibiotic was 99.9% pure.

Dilution and inoculums preparation

The leaf and root extracts of the plant; were dissolved in sterile distilled water to obtain concentration of 800µg/ml, 400µg/ml, 200µg/ml, 100µg/ml and 50µg/ml, 25µg/ml respectively. *Staphylococcus aureus* and *Shigella dysentriae* were prepared in nutrient broth medium and at 30°C for 24 h and the stock culture was maintained at 4°C and sub cultured as needed. Streptomycin 100µg/ml concentration was used for the standard drugs.

Procedure for performing disc diffusion test

Nutrient Agar media was prepared by the method given in standard procedure with slight modifications [6]. The prepared media was sterilized in an autoclave at 121°C for 15 minutes. In this technique, petridishes of Agar are prepared by pouring melted Agar media previously inoculated with selected microorganism. After the solidification of Agar cups are made with the help of borer and cups are filled with solution of suitable concentration of sample and standard respectively and are inoculated at 37ºC for 24 hours. The sterile discs were loaded with different concentrations of about 800µg/ml, 400µg/ml, 200µg/ml, 100µg/ml and 50µg/ml, 25µg/ml of plant extracts and antibiotic streptomycin into each separate disc of about 100µl. The bacterial strains obtained from (Microbiology Laboratory of Sunsari Technical College.) were used for evaluating antimicrobial activity. The bacterial stock cultures were incubated for 24 hrs at 37°C on Nutrient Agar media following refrigeration storage at 4°C. The bacterial strains were grown in Mueller–Hinton agar (MHA) plates at 37°C, The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C). The discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to allow good diffusion and then transferred to an incubator at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader.

Antihelmintic activity of ethanolic extracts of leaves

The anthelmintic activity was performed according to the method with slight modifications [7]. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors.

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil, and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2cm in width were used for all the experimental protocol. The different concentration of plant extracts were prepared in distilled water (which served as negative control). 10mg/ml, 25mg/ml, and 50mg/ml for ethanolic extracts of leaves were administered to each disc separately.

The standard (praziquantel) drug was prepared in distilled water at a dose level of 10mg/ml. The earthworm which served as normal control received distilled water only. Then the earthworm received the standard drug i.e. Praziquantel at a dose level of 10mg/ml and remaining earthworm received different concentration of 10mg/ml, 25mg/ml & 50mg/ml ethanolic extracts of leaves. Observations were made for the time taken to cause paralysis and death of individual worms. Paralyzing and death time was concluded when the worms lost their motility followed with fading away of their body colors.

RESULTS & DISCUSSIONS

Macroscopical studies:

General appearance:

Leaves	1.7 cm long pinnate leaves round and globular.
Flowers	The flowers consist of half inch diameter five petals and pale yellow in color
Roots	Hard, Cylindrical root, 1.2-1.4 cm in thickness, slightly ribbed, gradually tapering, yellowish-brown to dark brown in color.

Organoleptic properties:

***Odor*:** Leaves had pungent and root had characteristic odor

Color: Leaves were green in color.

Phytochemical screening

Phytochemical screening of the plant showed the presence of different constituents in different solvent extracts. The phytochemical analyses showed that the various groups that were found to be present in the different extracts are listed in **Table no 3.**

Thin layer chromatography

Chromatography is an important technique to identify the formulation of new compound and also to determine the purity of new compounds. The R_f value is characteristic of each

compound. Thin layer chromatographs of different compound were carried out by preparing glass plates using Silica gel slurry. The plates were then dried in hot air oven and drops of each extract were placed in different plates and the plates were kept in beaker containing the appropriate solvent followed by calculation of R_f value.

Table No 3: Phytochemical Test of plant extracts

Tests	Aqueous leaves	Aqueous roots	Ethanolic leaves	Ethanolic roots
Alkaloids				
Mayer's test	-	-	-	-
Wager's test	-	-	-	-
Hager test	-	-	-	-
Dragendroff test	-	-	-	-
Carbohydrates				
Molish's test	+	+	+	+
Benedicts test	-	-	-	-
Fehlings test	-	+	-	-
Glycosides	+	+	+	+
Saponins	+	+	+	+
Phytosterols (Salkawoski's test)	-	+	+	+
Phenols	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	-	+
Proteins	+	-	+	+
Diterpenens (copper acetate test)	+	+	+	+

Table No 4: TLC of extract

Extract	Solvent system	Rf value
Leaves	Distilled Water	0.615
Roots	Distilled water	0.552
Leaves	Ethanol	0.831
Roots	Ethanol	0.664

Table No 5: Zone of inhibition (mm) of aqueous leaves extracts vs standard

Bacterial strains	Gentamicin 100µg/ml	Plant extract concentration(µg/ml)					
		800	400	200	100	50	25
Staphylococcus aureus	2.7	2	1.7	1.3	1.2	1	-
Citrobacter 4koseri	2	1.7	1.5	1.3	1.0	0.7	-

Table No 6: Zone of inhibition (mm) of ethanolic leaves extracts vs standard

Bacterial strains	Gentamicin 100µg/ml	Plant extract concentration(µg/ml)					
		800	400	200	100	50	25
Staphylococcus aureus	2.7	1.5	1.3	1.0	-	-	-
Citrobacter koseri	2	1.4	1.1	1.0	-	-	-

Table No 7: Zone of inhibition (mm) of aqueous roots extracts vs standard

Bacterial strains	gentamicin 100µg/ml	Plant extract concentration(µg/ml)					
		800	400	200	100	50	25
Staphylococcus aureus	2.7	2	1.7	1.5	1.1	0.8	-
Citrobacter koseri	2	1.8	1.7	1.6	1.4	1.1	-

Table No 8: Zone of inhibition (mm) of ethanolic root extract vs standard.

Bacterial strains	gentamicin100µg/ml	Plant extract concentration(µg/ml)					
		800	400	200	100	50	25
Staphylococcus aureus	2.7	2	1.7	1.5	1.3	1.0	-
Citrobacter koseri	2	1.5	1.4	1.3	1.0	-	-

Table No 9: Antihelmintic activity of leaves extracts

Plant extracts	Antihelmintic activity		
	Concentration(mg/ml)	Paralyzing time(min)	Death time(min)
Ethanolic Leaves extract	15mg/ml	28	90
	25mg/ml	24	80
	50mg/ml	20	70
Paziquantel	10mg/ml	5	20
Distilled water	-	-	-

This study reveals that Preliminary phytochemical screening of alcoholic extract of leaves and roots has the presence of glycoside, phenolic compounds, tannins, and diterpenes while aqueous extract showed the presence of all the phytochemical constituents so these results showed the presence of these secondary metabolites. It has been reported that alcoholic extract of leaves and roots has the presence of glycoside, phenolic compounds, tannins and diterpenes [8]. Ethanolic extract and aqueous extract of *Cassia tora* showed antibacterial activity against all tested bacteria but maximum activity were showed by all extract against gram positive bacteria *Staphylococcus aureus.*

Phytochemical analysis of the crude extracts revealed presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show anthelmintic activity [9]. Previous reports revealed that some synthetic phenolic e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [10]. Here ethanolic leaf extracts were subjected to antihelmenthic activity against standard drug praziquantel and the activity was found to be moderate.

CONCLUSION

Cassia *tora* is the most valuable plant which has various pharmacological activities that has been reported earlier. This study reveals that aqueous extract gives more yield value than the ethanolic solvent. The Preliminary phytochemical screening of alcoholic and aqueous extract of leaves and roots showed the presence of glycoside, phenolic compounds, tannins and diterpenes except alkaloids. Ethanolic extract of leaves exhibited anthelminthic activity which paralyses the worm. Antibacterial activity was found better in all extract against gram positive bacteria. *Staphylococcus aureus*in comparison to gram negative bacteria. The resultprovides a support for the use of Cassia *tora* leaves and roots in traditional medicine and suggests its future advance investigation. Therefore further isolation identification of chemical constituents responsible for these activities as well as pharmacological and toxicological studies and are needed to explore the findings.

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CONFLICT OF INTEREST
The authors declare no conflict of interest

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