Original Article

Phytochemical Analysis and Assessment of *In-vitro* Anthelmintic Activity of *Cassia auriculata* Linn leaves

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ABSTRACT

Three different extracts namely Ethanol, Chloroform, Petroleum ether of *Cassia auriculata* Linn (Family: Caesalpiniaceae) leaves were screened for their phytochemical composition. The phytochemical studies of *Cassia auriculata* leaves show the presence of alkaloids, tannins flavonoids, glycosides, saponins along with proteins. Three extracts viz. Petroleum ether, Methanol and Chloroform extracts of *Cassia auriculata* leaves were investigated for the anthelmintic activity against earthworms [*Megascoplex konkanensis*]. Three concentrations [20, 40, 60mg/ml] of each extract were studied which included the determination of time of paralysis and time of death of earthworms. Albendazole [10 mg/ml] was used as standard drug and distilled water containing 2% Tween 80 was used as control. All the extracts exhibited dose dependent anthelmintic activity. The decreasing order of activity of extracts was assessed to be Methanol, Petroleum ether, and Chloroform.

Keywords: *Cassia auriculata*, Anthelmintic, Albendazole.

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency[1]. The cost of drugs in use today is too expensive for the majority of the population in the third world countries and therefore the search for some cheap sources of antimicrobial substances in nature become inevitable. Plants are good

sources for new safe, biodegradable and renewable drugs. The use of plants as therapeutic agents in addition to being used as food is age long. Though the therapeutic uses of plants by the primitive people lack scientific explanations[2], there is a great awareness in the use and significance of these medicinal floras by the World Health Organization in several resource- poor nations[3]. This has led to intensified efforts on the documentation of medicinal plants[4]. *Cassia auriculata* Linn (Family: Caesal-piniaceae) commonly known as *Tanners Senna*, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine. The plant has been reported to possess antipyretic[5], hepato-protective[6], antidiabetic, antiperoxidative and antihyperglyceamic[7] and microbicidal activity[8]. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation[9]. They are one of the constituent of polyherbal formulation 'Diasulin' in the concentration range of 40 mg/dl which is proven to have antidiabetic activity[10].

MATERIALS AND METHODS

Collection and Identification of Plant material

Cassia auriculata leaves were collected freshly from Moradabad District, Uttar Pradesh, India in the month of January. The plant was identified and authenticated at the Herbarium of Botany Department in MET Faculty of Pharmacy Moradabad and voucher specimen (SGPG 284) was deposited.

Extraction and Isolation

The collected leaves were shade dried and then powdered to the 22 mesh size and stored in an airtight container. The powdered leaves about 20 gms of *Cassia auriculata* were subjected to Soxhlet extraction at room temperature for 48 hours in 2.5 L of ethanol. The mixture was filtered using Whatman no - 1 filter paper and the filtrate solution was concentrated and evaporated on a rotary evaporator (Buchi, R–124 and Switzerland) to obtain a residue which was used for further investigations. The same procedure was repeated to obtain the Chloroform, Petroleum ether (40-60ºC) extract of plant leaves.

Preliminary Phytochemical Analysis

The preliminary phyto-chemical studies were performed to identify the presence of different chemical groups present in all the three extracts[11,12]. The following chemical group tests were performed and the results are tabulated in table 1.

Alkaloids

(a) Dragendroff's test

To 2 mg of the extract, 5 ml of distilled water and 2 M hydrochloric acid were added until an acid reaction occured. To this 1 ml of Dragendroff's reagent was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

(b) Hager's test

To 2 mg of the extract taken in a test tube, few drops of Hager's reagent were added. Formation of yellow precipitate confirmed the presence of alkaloids.

(c) Wagner's test

2 mg of extract was acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.

(d) Mayer's test

Few drops of Mayer's reagent were added to 2 mg of the extract. Formation of white or pale yellow precipitate indicated the presence of alkaloids.

Carbohydrate

(a) Molisch's test

In a test tube containing 2 ml of extract, 2 drops of freshly prepared 20% alcoholic solution of α-naphthol was added. 2 ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red-violet ring appeared which disappeared on the addition of excess of alkali, indicating the presence of carbohydrates.

(b) Anthrone test

To 2 ml of anthrone reagent solution, 0.5 ml of extract was added. Formation of green or blue colour indicated the presence of carbohydrates.

(c) Benedict's test

To 0.5 ml of extract, 5 ml of Benedict's solution was added and boiled for 5 minutes. Formation of brick red coloured precipitate indicated the presence of carbohydrates.

(d) Fehling's test

To 2 ml of extract, 1 ml of mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of red or brick red coloured precipitate indicated the presence of reducing sugars.

Cardiac Glycoside

(a) Keddes test

To 2mg extract add one drop of 90% alcohol and 2 drop of 2% 3, 5di- nitro benzoic acid in 90% alcohol made alkaline with 20%NaOH solution. Forming purple colour indicated the presence of glycoside.

(b) Keller killiani test

Take 2mg extract and add 0.40 ml glacial acetic acid containing a trace ferric chloride. Transfer carefully 0.5ml of concentrated H_2SO_4 by the side of the test tube. A blue colour appears in acetic acid layer indicated the presence of cardiac glycoside.

Flavoniods

(a) Shinod's test

In a test tube containing 0.5 ml of the extract, 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

(b) Zn-HCl reduction test

To the test solution add a mixture of zinc dust and concentrated hydrochloric acid. It gives red colour after few minutes, indicated the presence of flavonoids.

Triterpenoids

Libermann - Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicated the presence of triterpenoids.

Proteins

(a) Biuret test

To 1 ml of extract, 5-8 drops of 10% w/v sodium hydroxide solution, followed by 1 or 2 drops of 3% w/v copper sulphate solution were added. Formation of violet red colour indicates the presence of proteins.

(b) Millon's test

1 ml of extract was dissolved in 1 ml of distilled water and 5-6 drops of Millon's reagent were added. Formation of white precipitate, which turns red on heating, indicates the presence of proteins.

Resins

1 ml of extract was dissolved in acetone and the solution was poured in distilled water. The development of turbidity indicates the presence of resins.

Saponins

In a test tube containing about 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicates the presence of saponins.

Steroids

(a) Libermann-Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids.

(b) Salkowski reaction

2 mg of dry extract was shaken with chloroform and to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicates the presence of steroids.

Tannins

To 1-2 ml of the extract, few drops of 5% w/v ferric chloride solution were added. Formation of green colour indicates the presence of gallotannins, while brown color indicates the presence of pseudotannins.

Starch

01 g of Iodine and 0.075 g of potassium iodide were dissolved in 5 ml of distilled water and 2-3 ml of extract was added. Formation of blue color indicates the presence of starch.

Anthelmintic Activity

Methanolic, chloroform and petroleum ether extract of *Cassia auriculata* were tested for the anthelmintic activity against *Megascoplex konkanensis* (earth worms). Three different concentrations (20, 40 and 60mg/ml) of each extracts were used to evaluate the time of paralysis and time of death of earthworms. Albendazole, 10mg/ml was taken as standard reference and distilled water (2% tween 80) as control. The anthelmintic assay was carried out by Garg's method. Indian adult earthworms collected from herbal garden of the institute were washed with normal saline to remove all faecal matter, were used for anthelmintic study. The earthworms of 4-6 cm in length were used for all the experimental protocol. Test samples of the extract was prepared at concentrations 20-100mg/ml in distilled water and six earthworms approximately equal size were released in 25 ml test solutions. All the test solutions and standard drug solution were prepared freshly before starting the experiment. 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 colours. All experiments were carried out in accordance with the guidelines of the institutional biosafety and ethical committee.

RESULT AND DISCUSSION

Phytochemical Screening

In the Preliminary phytochemical analysis of the *Cassia auriculata* leaves it was revealed that methanolic extract was found to show the presence of Alkaloids, Carbo-hydrates, Glycosides, Flavonoids, Resins, Saponins, Proteins and Tannins, the petroleum ether extract was found to have

Alkaloids, Carbohydrates and Saponins, where as Alkaloids and Carbohydrates were present in Chloroform extract.

Biological Activity

As reported in the tables 2. All the extracts exhibited dose dependent anthelmintic activity against earthworms. *Cassia auriculata* leaves extracts showed significant effects ($P<0.05$) at the tested concentrations [20-60 mg/ml] as determined by the paralysis and death time. Methanolic extract was found to be most effective in causing paralysis and death of earth worm at all concentrations. The decreasing order of anthelmintic activity of different extracts taken may be arranged as methanolic > Petroleum ether > Chloroform extracts. Methanolic extract exhibits better anthelmintic activity than standard at 40-60mg/ml dose. In case of petroleum ether extract, paralysis was caused earlier but death time was longer. The anthelmintic activity of *Cassia auriculata* leaf extract may be due to the presence of tannins and phenolic compounds. Tannins produce anthelmintic activity by binding to free protein in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of the parasite and phenolic compounds by uncoupling oxidative phosphorrylation hinder the energy production in helminth parasites[13,14]. Phytochemical analysis of leaves of *Cassia auriculata* revealed the presence of tannins and phenolic as constituents. Further study is to be done to determine the mechanism involved and constituent responsible for anthelmintic property.

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Table 1. Preliminary Phytochemical Analysis of the Ethanol, Chloroform and Petroleum ether extracts of *Cassia auriculata* Leaves

S. No	Constituents	Tests	Inference		
			Methanolic	Pet. ether	Chloroform
1.	Alkaloids	Dragendroff' test	+ve	-ve	+ve
		Hager's test	+ve	-ve	+ve
		Wagner's test	+ve	-ve	+ve
		Mayer's test	+ve	-ve	+ve
2.	Carbohydrates	Anthrone test	+ve	+ve	+ve
		Benedict's test	+ve	+ve	-ve
		Fehling's test	+ve	+ve	-ve
		Molisch's test	+ve	+ve	-ve
3.	Starch	Iodine test	-ve	-ve	-ve
4.	Glycosides	Keddes test	+ve	-ve	-ve
		Killer killani test	+ve	-ve	-ve
5.	Flavonoids	Shinoda's test	+ve	-ve	-ve
		Lead acetate test	+ve	-ve	-ve
		Ferric chloride test	+ve	-ve	-ve
6.	Triterpenoids	Libermann Burchard's test	-ve	-ve	-ve
7.	Resins		+ve	-ve	-ve
8.	Saponins		+ve	+ve	-ve
9.	Steroid	Libermann Burchard's test	-ve	-ve	-ve
		Salkowaski reaction	-ve	-ve	-ve
10.	Proteins	Millon test	+ve	-ve	-ve
		Biuret test	+ve	-ve	-ve
11.	Tannins	Ferric chloride test	+ve	-ve	-ve

Table 2. Anthelmintic activity of *Cassia auriculata* leaves extract

	Dose (mg/ml)	Paralysed time (min) Mean±SEM	Death Time (min) Mean±SEM
Control	0	Nil	Nil
Albendazole	10	25.3±0.88	70.67±0.67
Petroleum ether extract	20	25.0±0.68**	98.5±0.88**
	40	18.2±0.68**	86.0±1.0**
	60	15.6±0.67**	52.7±0.88**
Methanolic extract	20	32.7±1.25**	46.2±1.0**
	40	16.8±1.64**	23.0±0.58**
	60	10.5±0.27**	19.7±0.82**
Chloroform extract	20	82.3±1.05**	120.7±0.67**
	40	32.3±1.45	96.0±0.58**
	60	20.3±0.87**	79.7±0.32**