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### Investigation of *in vitro* anthelmintic activity of *Cassia auriculata* leaves

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#### ABSTRACT

Four extracts viz. Petroleum ether, Ethyl acetate, Ethanol and Aqueous extracts of *Cassia auriculata* leaves were investigated for the anthelmintic activity against earthworms [*Eisenia foetida*]. Three concentrations [20, 40, 60 mg/ml] of each extract were studied which included the determination of time of paralysis and time of death of earthworms. Piperazine citrate [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 ethyl acetate, ethanol, petroleum ether and aqueous extracts.

**Keywords:** *Cassia auriculata*, *Eisenia foetida*, anthelmintic, Piperazine citrate

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#### INTRODUCTION

Helminthiasis is a macroparasitic disease observed in humans and animals in which a part of the body is infested with parasitic worms such as Roundworms [Nematodes], Tapeworms [Cestodes] or Flukes [Trematodes]. Typically, the worms reside in the GI tract but may also burrow into other organs like liver [*Fasciola hepatica*], lung [*Paragonimus westermani*], muscle [cysticercosis], skin [Strongyloides], lymph [*Wuchereria bancrofti*], eye [*O. volvulus*], brain [*Paragonimus sp*] and other tissues. Anthelmintics are drugs that destroy or expel parasitic intestinal worms from the body, by either vermifuges [stunning] or vermicides [killing]. Most of the existing anthelmintics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea [1]. Since ancient times herbal drugs are used for the treatment of parasitic diseases in human without any side effects [2]. To eradicate the side effects of the present allopathic drugs now scientist are moving towards the herbal drugs what our ancient peoples used. The present study was carried out to determine the effects of petroleum ether, ethyl acetate, ethanol, and aqueous extracts of *Cassia auriculata* leaves as anthelmintic activity [30].

*Cassia auriculata* L. [family- Ceasalpinaceae] is a shrub with large bright yellow flowers found growing wild in central and western India and cultivated in other areas of the country. The tribals of the Chittoor district of Andhra Pradesh use this plant for the treatment of skin diseases, asthma, conjunctivitis and renal disorders [3]. In the folk medicine, tea prepared from the leaves is useful in chronic fever and fruits are used as anthelmintic [4]. *Cassia auriculata* has been used for a variety treatment like in case of leucorrhoea [5], scorpion bite [6] etc. The 50% ethanolic extract of flower and leaves is reported to have antiviral, antispasmodic, antilipidemic [7] and antipyretic

activity [8]. The plant has been also reported a variety of medicinal uses such as antidiabetic [9], anticancer [10], hepatoprotective [11], antirheumatic, antiasthmatic activities etc. [4].

### Traditional uses

#### Leaves and fruits

The tea prepared from the leaves is used in chronic fever and fruits are used as anthelmintic [4]. Tribals of Eastern Ghats of Andhra Pradesh, make pills from ground leaves and fruits, which are given orally with limewater for 3 days for the treatment of leucorrhoea [5]. It was observed that the Southern Indian tribals prepare paste from leaves in vinegar, which applied on skin for various skin diseases [12, 13]. In Gundlabrahmeswaram Wild Life Sanctuary, Andhra Pradesh; tribals dropped the juice of fresh macerated leaves into ears in case of scorpion bite [6]. It was also found that the tenders of leaves mixed with lime and is given once a day for treatment of stomachache [14]. Ganesan *et al.*, 2009 reported that leaves and flowers are used for treatment of diabetes and for religious function in sacred grove of Pallapatty village [reserved forest], Madurai district, Tamilnadu [15].

#### Flowers

Flowers are used for spermatorrhoea [4]. The dried powder of flowers mixed with goat milk and taken orally to prevent white discharge in Kancheepuram district of Tamilnadu [16]. The flowers are also uses as food stuff by tribal people of Andhra Pradesh [17]. Flowers are mixed with whole plant of *Enicostema axillare* and fruits of *Cuminum cyminum*, given to cattle along with grass for three days prevent heat diseases [18].

#### Other parts

According to Ayurveda, the different parts of plant have been used for various ailments. Roots are useful in urinary discharges and cures tumours, skin diseases and asthma. Powder of bark is uses for fixing teeth and decoction for chronic dysentery. Decorticated seeds in fine powder and paste are valued local applications to purulent ophthalmia and conjunctivitis [4]. The tribals of the Chittor district of Andhra Pradesh used various parts of the plant for the treatment of skin diseases, asthma, conjunctivitis and renal disorders [3]. The tribals of Eastern Ghats of Andhra Pradesh uses whole plant and roots orally in form of pills with pinch of red soil for 3 days for Leucorrhoea [5]. Valaiyan community of Piranmalai hills, Tamilnadu flora tribal uses different part of *Cassia auriculata* in the treatment of body heat and cuts [19]. Tribal's mixed stem decoction, mixed with garlic and powdered pepper, given to cattle as purgative in Southern districts of Tamilnadu [20].

#### Collection of plant material and its identification

The leaves of *Cassia auriculata* was collected in the blooming season [July- Aug] in 2009, in the region of Ooty, Tamilnadu. Fresh *Cassia auriculata* leaves were authenticated by Dr. Rajan, Prof. and Head of Department of Botany, Government College of Ooty, Tamilnadu. The coarse powder was extracted with ethyl acetate, ethanol, petroleum ether and aqueous. The extract was distilled, dried with heating at temperature 50-60°C and extract stored in desiccators until further use.

#### Extracts used

Four extracts were used viz. petroleum ether, ethyl acetate, ethanol and aqueous extracts which were prepared by adopting the successive solvent extraction method using the Soxhlet apparatus.

#### Organism used

Adult earthworms [*Eisenia foetida*] were used and were procured and authenticated from Ujjwal Ujala Vermi Group, Amritsar.

#### Phytochemicals screening

The preliminary phytochemical screening of the aqueous extract of leaves of *Cassia auriculata* was carried for active constituents such as alkaloids, phenols, glycosides, flavonoids, tannins, saponins, proteins, carbohydrates and anthraquinone derivatives. The procedures to identify the presence of these constituents are as: Alkaloids [Dragendorff's reagent] - Alkaloids give reddish brown precipitate with Dragendorff's reagent [potassium bismuth iodide solution]. Glycosides - Extract 200 mg of drug with 5 ml of dilute sulphuric acid by warming on water bath. Filter it. Then neutralize the acid extract with 5% solution of sodium hydroxide. Add 0.1 ml of fehling's solution A and B until it becomes alkaline [test with pH paper] and heat on a water bath for 2 minutes. The formation of red precipitate indicates the presence of glycosides. Cardiac glycoside [Legal's test] - Treat the test solution with picric

acid or sodium picrate, orange colour is formed. Flavonoids [Shinoda test] -To the test solution add few magnesium turnings and concentrated hydrochloride acid dropwise, pink scalet, crimson red or occasionally green to blue colour appears after few minutes. Tannins [Ferric chloride test] - Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present. Proteins [Trichloroacetic acid test] - To the test solution add Trichloroacetic acid, precipitate is formed. Amino acids [Ninhydrine test] - To the test solution add Ninhydrine solution, boil, violet colour indicates presence of amino acid. Carbohydrates [Molisch's test] - To the test solution add few drops of alcoholic a-naphthol, then add few drops of concentrated sulphuric acid through sides of test tube, purple to violet colour ring appears at the junction. Steroids [Liebermann-burchard test] - Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction of two layers and upper layer turns green which shows presence of steroids [28].

#### Anthelmintic activity

Test samples of all four extracts were prepared at the concentrations, 20, 40 and 60 mg/ml in 25 ml of distilled water containing 2% Tween 80. Six earthworms of approximately same size were placed in petridish [diameter 9 cm] containing above solution of extracts. Piperazine citrate [10 mg/ml] was used as standard drug and distilled water containing 2% Tween 80 was used as control. Anthelmintic activity of Piperazine citrate mediates through hyper polarization that leads to muscle relaxation and flaccid paralysis [21]. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was noted when the earthworms neither moved when shaken vigorously or when dipped in warm water [50°C] [22].

### RESULTS AND DISCUSSION

The preliminary phytochemical screening of the aqueous extract of leaves of *Cassia auriculata* revealed the presence of biologically active ingredients such as alkaloids, phenols, glycosides, flavonoids, tannins, saponins, proteins, carbohydrates and anthraquinone derivatives. The chemical constituents of the test solution are represented in table 1.

As reported in the tables 2 and 3. All the extracts exhibited dose dependent anthelmintic activity against earthworms. *Cassia auriculata* leaf extracts showed significant effects [P<0.001] at the tested concentrations [20-60 mg/ml] as determined by the paralysis and death time [Table 2]. Ethyl acetate extract was most effective in causing death of earthworms at all concentrations. The decreasing order of anthelmintic activity of different extracts taken comes out to be - ethyl acetate > ethanol > petroleum ether extracts. Ethyl acetate extract exhibits better anthelmintic activity than the standard. In the case of petroleum ether extract, paralysis was caused earlier but death time was longer. In the case of ethanol extract, the paralysis time was longer at lower dose [20 mg/ml] but shorter at higher doses [40-60 mg/ml]. The death time was long but shorter than that of petroleum ether extract.

Indian earth worms resemble intestinal round worm parasite of human beings [23-24]. Tannins produce anthelmintic activity by binding to free protein in the gastrointestinal tract of the host animal [25] or glycoprotein on the cuticle of the parasite [26] and phenolic compounds by uncoupling oxidative phosphorylation hinder the energy production in helminth parasites [27]. 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 [29].

**Table: 1 Preliminary phytochemicals screening of aqueous extract of *Cassia auriculata* leaves**

Phytochemicals	Inference
Alkaloids	+
Phenols	+
Glycosides	+
Flavonoids	+
Tannins	+
Saponins	+
Proteins	+
Carbohydrates	+
Anthraquinone derivatives	+

[+] = Present

Table 2 Effects of control and standard drug on earthworms

Conc. [mg/ml]	Control		Standard	
	Paralysis time [min.]	Death time [min.]	Paralysis time [min.]	Death time [min.]
10	-	-	25.3±0.88	70.67±0.67

Table 3 Effects of *Cassia auriculata* leaf extracts on earthworms

Conc. [mg/ml]	Petroleum extract		Aqueous extract		Ethyl acetate extract		Ethanol extract	
	Paralysis time [min.]	Death time [min.]						
20	25.0±0.68**	98.5±0.88**	82.3±1.05**	120.7±0.67**	7.2±0.88**	12.7±0.88**	32.7±1.25**	46.2±1.0**
40	18.2±0.68**	86.0±1.0**	32.3±1.45	96.0±0.58**	5.0±0.58**	9.3±0.67**	16.8±1.64**	23.0±0.58**
60	15.6±0.67**	52.7±0.88**	20.3±0.87**	79.7±0.32**	2.5±0.29**	5.5±0.29**	10.5±0.27**	19.7±0.82**

Significant at \* $P < 0.05$ , \*\* $P < 0.01$  [One way ANOVA, Dunnet: compare all vs. standard applied] Standard vs. low, medium and high doses. Values are mean  $\pm$  SEM, n = 6.

### CONCLUSION

From the results it concludes that, aqueous extract of *Cassia auriculata* leaves demonstrate to possess dose dependant anthelmintic activity when compared to Piperazine citrate. The anthelmintic activity of *Cassia auriculata* leaves as found to be inversely proportional to the time taken for paralysis and time of death of the worms. The active constituents can be responsible for anthelmintic activity. The possible mechanism of the anthelmintics activity of *Cassia auriculata* cannot be explained on the basis of present results. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

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