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Phytochemical analysis and anthelmintic activity of stem of *Cassia auriculata linn*

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Abstract
The present investigation aimed to screen the phytochemicals in the ethanolic and chloroform stem extracts of *Cassia auriculata* and evaluate their anthelmintic activity by using adult Indian earthworm *Pheretima posthuma*. Phytochemical screening of the extracts revealed the presence of Carbohydrates, Flavonoids, Phenols, Amino acids, Glycosides, Saponins, Fixed oils, Alkaloids, Phytosterols and Tannins. Chloroform and ethanolic extract of *Cassia auriculata* were investigated for anthelmintic activity using Albendazole (10 mg/ml) as standard reference and normal saline as control. The time to achieve paralysis and death of worm were determined. Chloroform extract shows more anthelmintic activity than ethanolic extract when compared with standard Albendazole.

Keywords: *Cassia auriculata*, *Pheretima posthuma*, Anthelmintic activity

Introduction
Helminthiasis or infection with parasitic worm is pathogenic for human beings. Immature form of the parasites invade human beings via the skin or gastrointestinal tract and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelmintics are drugs that act locally to expel worms from the GIT or systematically to eradicate adult helminths or development forms that invade organs and tissues. Most of the existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea [1]. Chemotherapy is the only treatment and effective tool to cure and control helminths infection, as effective vaccines against helminths have not been developed so far. Indiscriminate use of synthetic anthelmintics may lead to resistance of parasites [2]. Helminthic infections are not limited to human beings; they infect a wide range of livestock leading to reduced productivity and mortality [3]. Thus they have major health as well as economic implications. The World Health Organization WHO has recommended various drugs such as Mebendazole, Albendazole, Ivermectin and Levimisole to tackle helminthiasis [4]. A major setback in the use of these drugs is development of genetic resistance [5] in the parasites as seen in case of Levimisole [6], Albendazole, Ivermectin and Mebendazole [7]. Thus there is a need to develop alternative drugs that can tackle worm infestations effectively. Analyses of various traditional medical practices have indicated the use of herbal preparations for worm infestations [8] which need to be scientifically explored.
Cassia auriculata Linn belongs to the family Caesalpiniaceae [9] and it is distributed throughout central and south India, most district on dry stony hills and black cotton soil. It is tall, branching shrub, flowers are large yellow in color. The phytochemical studies revealed the process of flavonoids and alkaloids. It is used in traditional system of medicine for antidiabetic, anthelmintic, antimicrobial and anti-inflammatory agent [10]. No systematic studies on anthelmintic activity have been made to establish the anthelmintic activity in this plant and its related species. Hence steps taken whether this plant process the above property and to check other phytochemical constituents.

Taxonomical classification of *Cassia auriculata* [11]

Kingdom	:	Plantae
Subkingdom	:	Tracheophytes
Order	:	Fabales
Family	:	Fabaceae
Genus	:	Senna
Species	:	Cassia auriculata
Tamil	:	Avarai
Malayalam	:	Arivam
Hindi	:	Tarvar

Materials and Methods

Plant material

The stem part of *Cassia auriculata* was collected from Tirunelveli district in October 2019 and authenticated by Dr. D. Amish Abraham, M.Sc, M.Phil, Ph.D., Head of the Department of Botany, St. John's College, Palayamkottai, Tirunelveli District. A voucher specimen of *Cassia auriculata* (SARPC/RXA/CC286) was deposited in department of Pharmaceutical Chemistry in S.A. Raja Pharmacy College, Tirunelveli for future reference. The air dried plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh and then stored in a air tight and light resistant container for further use

Fig 1: Morphology of *Cassia auriculata* Linn.

Fig 2: Stems of *Cassia auriculata* Linn.

Preparation of plant extracts

About 1kg of coarsely powdered stem of plant material was first extracted with chloroform for 72 hours. The extract was concentrated using rotary evaporator to get solid residue. The marc was removed, dried and successfully extracted with ethanol with hot percolation method until complete extraction was effected. It was then concentrated under reduced pressure and finally dried in dessicators. The percentage yield of each extract was calculated on dry weight analysis and all the extract were stored in dessicators for further phytochemical and pharmacological studies.

Qualitative Analysis

All the stem extracts were subjected to rout line Qualitative phytochemical analysis [12-14].

1. Test for carbohydrates

- **Molisch's Test**

A small amount of extract was treated with alpha-naphthol and concentrated sulfuric acid was added along the sides of test tube. Purple color obtained at the junction between two liquids which indicates the presence of carbohydrates.

- **Fehling's Test**

To the small amount of extract was treated with equal quantity of Fehlings solution A and B is heated gently in water bath for few minutes, brick red color precipitated was obtained which indicates the presence of carbohydrates.

- **Benedicts Test**

To 5 ml of benedict's reagent, added 8 drops of extract solution and mixed well, then boil the mixture vigorously for 2 minutes and then cooled. Red precipitate was obtained which indicates the presence of carbohydrates.

- **Barfoed's Test**

To 5 ml of Barfoed's reagent, added 8 drops of extract solution and mixed well, then boil the mixture for few minutes, A red precipitate indicates the presence of carbohydrates.

- **Test for Starch**

To 1 ml of extract solution, add 1 ml of dilute iodine solution. Bluish black color is produced which indicates the presence of starch.

2. Test for glycosides

- **Legals Test**

The extract was dissolved in pyridine and sodium nitroprusside solution was added and made alkaline. Pink color in organic layer shows the presence of glycosides.

- **Balijet Test**

To the extract, add sodium picrate an yellow to orange color shows the presence of glycosides.

- **Borntragers test**

A few ml of dilute sulfuric acid was added to the extract solution, boiled and filtered. To the filtrate, add chloroform, the organic layer was removed to which ammonia solution was added. Pink color in organic layer shows the presence of glycosides.

3. Test for phytosterols

- **Libermann Burchard test**

The extract was dissolved in 2 ml of chloroform in a dry test tube and add 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid. The solution becomes red, which later turns to bluish green color. It indicates the presence of sterols.

- **Salkowski Test**

Extract was dissolved in chloroform and added equal volume of concentrated Sulfuric acid. Cherry red color in chloroform layer and also green fluorescence, which indicates the presence of sterols.

4. Test for flavonoids

- **Shinoda's Test**

To the small quantity of extract was dissolved in alcohol and to this add magnesium metal followed by concentrated Hydrochloric acid is added in dropwise and heated. A magenta color was produced which indicates the presence of flavonoids.

- **Alkaline reagent test**

To the extract sodium hydroxide solution wad added. It gives yellow color which indicated the presence of flavonoids.

- **Mineral acid reaction test**

To the extract add concentrated sulfuric acid which gives orange color which indicates the presence of flavonoids.

- **Zinc HCL reduction test**

To the small quantity of extract, a pinch of zinc dust was added. Then add a few drops of concentrated Hydrochloric acid, magenta color was produced which indicates the presence of flavones.

- **Lead acetate solution test**

To a small quantity of the extract, 10% lead acetate solution was added. A yellow precipitate was product which indicates the presence of flavones.

Boric acid test

To 1 ml of solution of the extract add equal volume of boric acid solution. It gives yellow color which indicates the presence of flavonoids.

5. Test for fixed oil and fats

- **Spot test**

The extract was pressed on the filter paper, permanent oil appearance is produced on the filter paper which indicates the presence of fixed oil and fats.

- **Saponification test**

To the extract solution add potassium hydroxide solution and soap solution was formed which indicates the presence of fixed oil and fats.

6. Test for tannins and phenolic compounds

100mg of extract was boiled with 1ml of diluted water and filtered. The filtrate was used for the following test.

- **Ferric chloride test**

To 2ml of filtrate, 2ml of ferric chloride was added in a test tube. Formation of bluish color which indicates the presence of phenolic nucleus.

- **Lead acetate test**

To the 2ml of filtrate few drops of lead acetate solution was added in a test tube. Formation of yellow precipitate which indicates the presence of tannins.

- **Potassium dichromate test**

To the 1ml of filtrate add 15% of potassium dichromate a yellow color is produced which indicates the presence of tannins.

- **Potassium ferric cyanide test**

To 1ml of filtrate add potassium ferric cyanide followed by adding ammonia solution. Red color will be produced which indicate the presence of tannins.

7. Test for Protein and Amino acid

- **Biuret test**

To the extract solution add 1ml 10% sodium hydroxide and 2 drops of 1% copper sulphate, violet color will be produced which indicate the presence of proteins.

- **Ninhydrin test**

To the extract solution add 2 drops of freshly prepared 0.25% ninhydrin reagent and heated. A blue color indicates the presence of proteins.

- **Xanthoprotein test**

To the extract solution add conc. Nitric acid an orange color is produced which indicates the presence of aromatic amino acid.

- **Millon's test**

To the extract solution add 1ml millon's reagent. Red color produced which indicate the presence of proteins.

- **Tannic acid test**

To the extract solution add 10% tannic acid it gives white color is produced which indicates the presence of proteins.

8. Test for saponins

About one gram of the extract was boiled with 10ml distilled water for two minutes. The extract were filtered while hot and then cool. Collect the filtrate solution and the following tests were carried out.

- **Frothing test**

2.5ml of filtrate was shaken vigorously for 2 minutes with water. If any frothing was observed it indicates the presence of saponins.

- **Emulsifying test**

2.5ml of filtrate was shaken vigorously for few drops of olive oil. If an emulsifying layer is obtained which indicates the presence of saponins.

9. Test for alkaloids

Small quantity of the filtrate shaken vigorously with a few drops dil.Hydrochloric acid and filtered. The filtrate treated with various reagents such as Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent.

- **Wagner's test**

To 1ml of the filtrate few drops of wagner's reagent was added. It gives reddish brown precipitate produced which indicates the presence of alkaloid.

- **Dragendroff's test**

To 1ml of the filtrate few drops of dragendroff's reagents was added, orange color produced which indicate the presence of alkaloid.

- **Mayer's test**

To 1ml of filtrate few drops of Hager's reagent was added. It gives cream precipitate, which indicates the presence of alkaloid.

Test for coumarin

- **UV Fluorescence test**

The extract was dissolved in alcohol and exposed to UV light. It shows green fluorescence which indicates the presence of coumarins.

- **Ferric chloride test**

The extract was dissolved in alcohol and with ferric solution, a green color was obtained which indicates the presence of coumarins.

Anthelmintic activity

The anthelmintic activity was evaluated on the earthworms by the method of Mathew and Dash [15]. The assay was prepared on adult Indian earthworm due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings [16-18]. Equal sized (8±1cm) worms were selected for the study. The worms were washed with normal saline to remove all extragenous matter. Eight group of approximately equal size Indian earthworms consisting of six earth worms in each group were released into 5 ml of desired formulations. Each group was treated with one of the following. The first group served as control (received 1% gum acacia in normal saline), second group served as standard (received albendazole 10 mg/ml), third, fourth, and fifth group received chloroform extract 10, 25, 50 mg/ml in 1 % gum acacia in normal saline) and 6th,7th,8th group (received ethanol extract, 10 mg, 25 mg, and 50 mg/ml in 1% gum acacia in normal saline respectively). The above prescribed dose was prepared and poured into respectively labelled petri dishes and the volume was made up to 50 ml with normal saline. The standard Albendazole (10 mg/ml) and the test extract chloroform and ethanol extract (10 mg, 25 mg, 50 mg/ml) were evaluated for anthelmintic activity. The treatment protocol was depicted below.

Observations were made for the time taken for paralysis and death of individual worms. The mean paralysis time and mean Lethal time of standard and each extract was recorded. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded. When worms lost their motility followed by fading of their body color [19] (Grime., 2006). Death was also confirmed by dipping the worm in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement has ceased [20] (Temgenmongla., 2005). The results were depicted.

Treatment protocol

Group 1: Served as control (received 1% gum acacia in normal saline).
Group 2: Served as standard (received Albendazole 10 mg/ml).
Group 3: Served as treatment control group and was administered chloroform extract of *Cassia auriculata* (10 mg/ml) in 1% gum acacia in normal saline.
Group 4: Served as treatment control group and was administered chloroform extract of *Cassia auriculata* (25 mg/ml) in 1% gum acacia in normal saline.
Group 5: Served as treatment control and was administered ethanolic extract of *Cassia auriculata* (50 mg mg/ml) in gum acacia in normal saline.
Group 6: Served as treatment control group and was administered ethanolic extract of *Cassia auriculata* (25 mg/ml) in gum acacia in normal saline.
Group 7: Served as treatment control group and was administered ethanolic extract of *Cassia auriculata* (25 mg/ml) in 1% gum acacia in normal saline.
Group 8: Serves as treatment control group and was administered ethanolic extract of *Cassia auriculata* (50 mg/ml in 1% gum acacia in normal saline.

Table 1: Anthelmintic activity of CECA and EECA of *Cassia auriculata*

Group	Treatment	Time taken for paralysis (min)	Time taken for dead Ⅰ (min)
I	Control	-	-
II	Albendazole (100mg/ml)	42.16±0.25	69.52±0.27
III	Chloroform extract (10mg/ml)	50.30±0.31	83.64±0.36
IV	Chloroform extract (25mg/ml)	32.76±0.22***	56.86±0.32***
V	Chloroform extract (50mg/ml)	24.39±0.16***	42.88±0.21***
VI	Ethanol extract (10mg/ml)	70.19±0.24	118.7±0.32
VII	Ethanol extract (25mg/ml)	56.72±0.18	111.1±0.30
VIII	Ethanol extract (50mg/ml)	38.82±0.21	77.44±0.60

Values are significantly different from albendazole treated group ***p<0.001(one way ANOVA followed by Dunnett's test)

Statistical analysis

Results are expressed as mean ± SEM were evaluated by one way ANOVA followed by Newman Kew's multiple range tests. Values of p<0.01 were considered statistically significant.

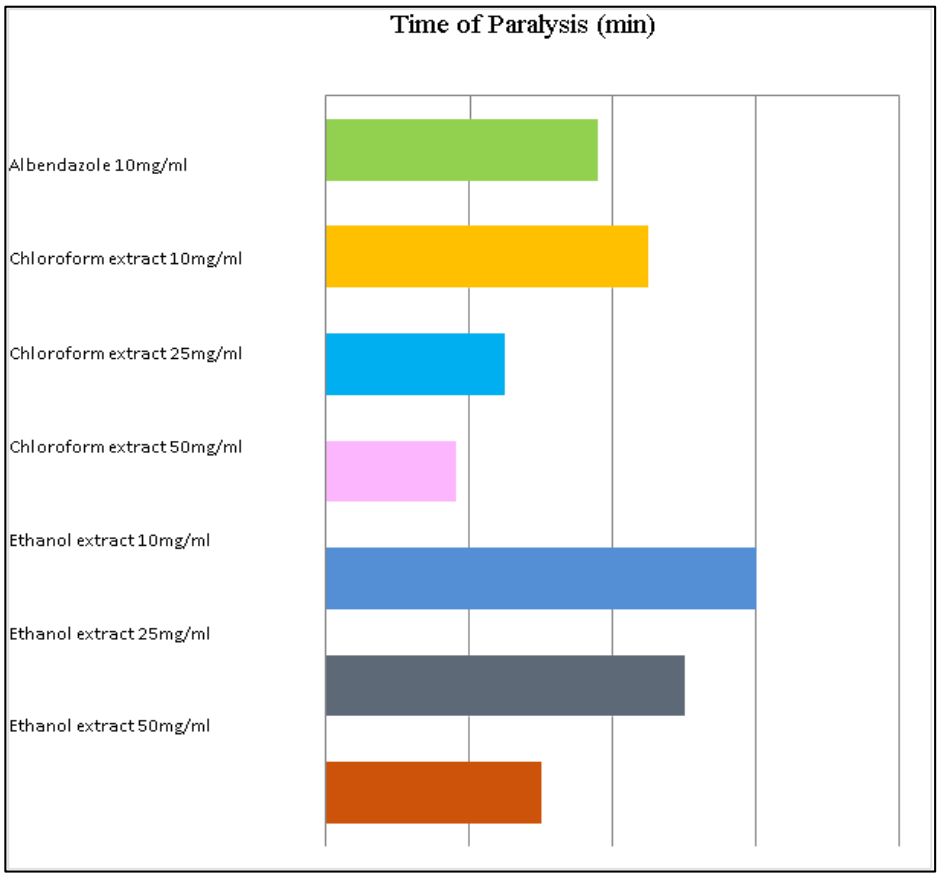

Fig 3: Effect of CECA and EECA on Time for Paralysis (min)

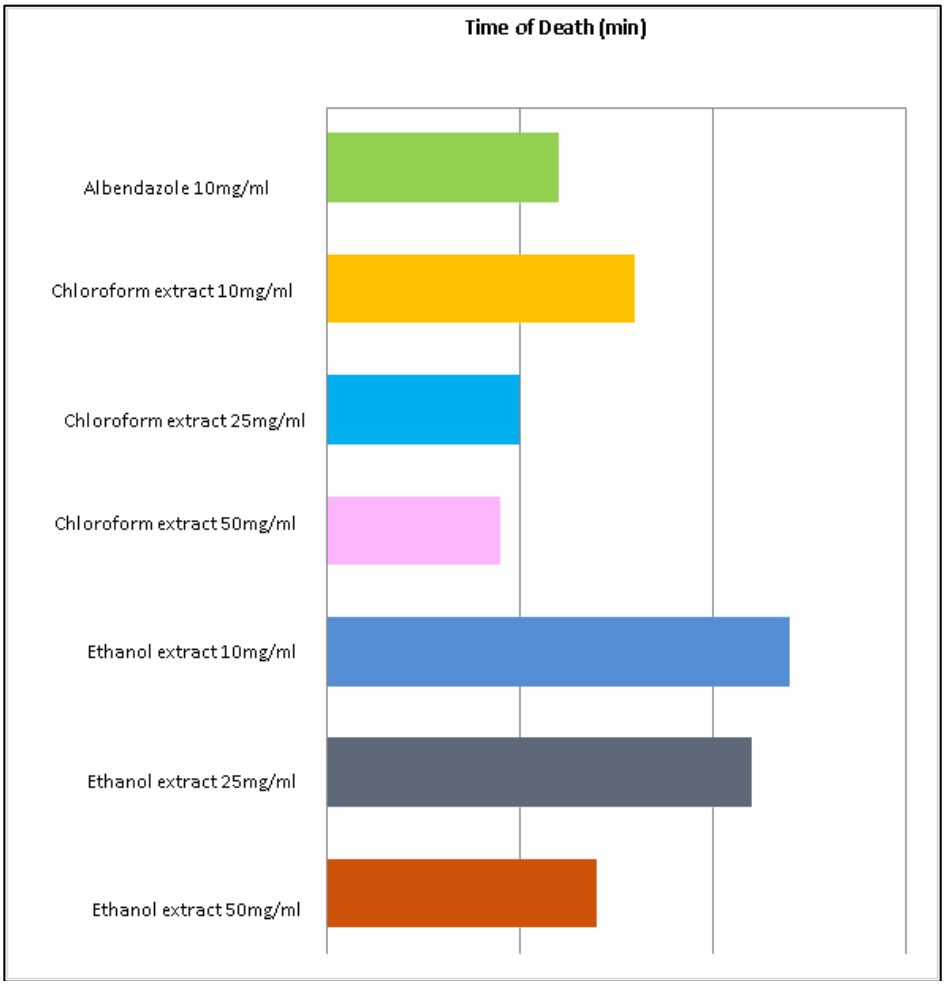

Fig 4: Effect of CECA and EECA on Time of Death (min)

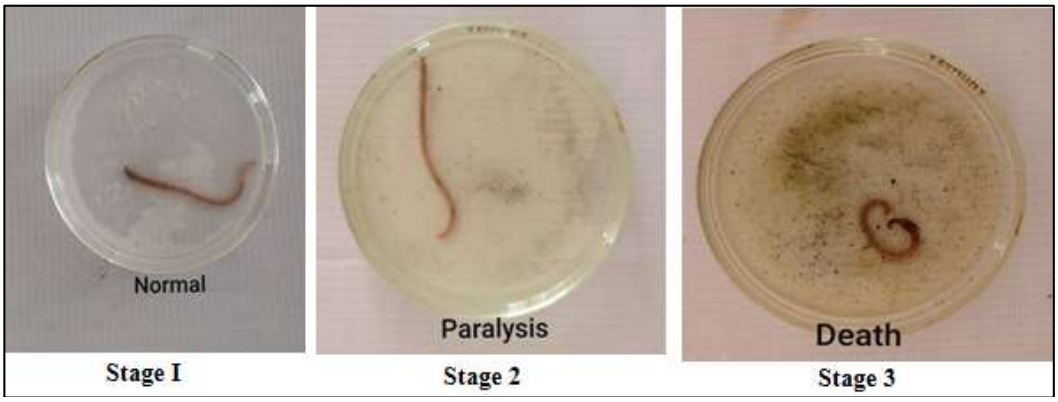

Fig 5: Different stages in anthelmintic activity

Results and Discussion

The anthelmintic activity was evaluated on Indian earthworm (*Pheretima posthuma*). The chloroform extract and ethanol extract showed good anthelmintic activity and the activities were compared with the effect produced by the reference standard drug Albendazole. The different extracts exhibited anthelmintic activity in dose different manner giving shortest time of paralysis and death with 50mg/ml within 24.39 minutes and time of death within 42.88 minutes while ethanol extract revealed paralysis with 38.82 minutes and time of death within 77.44 minutes against the earth worm *Pheretima posthuma.* The reference drug Albendazole showed the paralysis within 42.16 minutes and time of death 69.52 minutes respectively. The predominant effect of Albendazole on worm is to cause a flaccid *Cassia auriculata* Linn. Not only demonstrated paralysis but also caused death of worms especially at higher concentration of 50mg/ml in shorter time as compared to reference drug Albendazole.

The results of this work is limited to inability to report the actual compounds responsible for the activity reported because they were not isolated or investigated. However certain intermediate polar constituents may be responsible for anthelmintic activity than polar constituents.

Conclusion

The preliminary phytochemical analysis indicated the plant *Cassia auriculata* stem extracts contains carbohydrates, phenols, saponins, fats and fixed oils, alkaloids, Coumarins, glycosides and aminoacids. The chloroform and ethanolic extracts of *Cassia auriculata* Linn have anthelmintic activity. This justifies the use of the plant in folklore remedies as an anthelmintic drug of a natural origin. The traditional use of plant *Cassia auriculata* Linn as a powerful anthelmintic have been confirmed as the chloroform extract displayed profound anthelmintic activity in the study. Further studies are required to isolate the possible phytoconstituents which may be responsible for the anthelmintic activity and to explore the possible mechanism of action.

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Conflicts of Interest

The authors declare me conflicts of interest

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