

# Preliminary Analysis of Phytoconstituents and Evaluation of Anthelmintic Property of *Cayratia auriculata* (In Vitro)

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## ABSTRACT

**Context:** Phytoconstituents of medicinal plants have been playing a key role in treating various diseases all over the world since ancient times. The present study was focused on preliminary analysis of phytoconstituents and evaluation of anthelmintic property (in vitro) of different extracts of *Cayratia auriculata* (Family: Vitaceae) against earthworms (*Pheretima posthuma*).

**Materials and methods:** Four different hexane, chloroform, ethyl acetate and methanol extracts of *Cayratia auriculata* were screened for their phytoconstituents. *Cayratia auriculata* has been shown to have various phytoconstituents such as flavonoids, phenolic compounds, tannins, alkaloids, saponins, glycosides and steroids. Hexane, chloroform, ethyl acetate and methanol extracts of *Cayratia auriculata* were analyzed for their anthelmintic property on earthworms (*Pheretima posthuma*). Each extract at three different concentrations (20 mg/mL, 40 mg/mL and 80 mg/mL) was analysed to evaluate the time taken for paralysis (P) and death (D) of adult earthworms. Albendazole was used as standard and 2% Tween 80 in distilled water as control at a concentration of 10 mg/mL.

**Results:** Our study found that the methanolic extract of *Cayratia auriculata* at a concentration of 80 mg/mL [21.98±0.88 (P), 79.06±0.87(D)] expressed a stronger anthelmintic property than the standard albendazole (10 mg/mL) [26.73±0.56 (P), 87.70±0.91 (D)].

**Conclusion:** All extracts expressed a dose dependent anthelmintic activity, with an increasing pattern in the following order: hexane, chloroform, ethyl acetate and methanol.

**Keywords:** *Cayratia auriculata*, *Pheretima posthuma*, anthelmintic, albendazole.

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## INTRODUCTION

Natural products derived from medicinal plants with potent bioactive principles are the chief elements of phytomedicine. They have versatile applications in treating chronic as well as infectious diseases affecting mankind, which shows the immense potential of medicinal plants for use in various conventional systems (1). In modern medicine, highly marketed drugs such as artemisinin, quinine, and emetine are the best examples of pharmaceuticals derived from medicinal plants (2). Most of the drugs used in modern practice are very expensive for people living in developing countries, which gives a valuable tool for researchers to search for cheaper antibacterial substances from natural sources. Plants are the best natural sources for new safe, environment friendly and renewable drugs. Given the lack of scientific explanations for the therapeutic uses of medicinal plants by primitive peoples, a careful and well conducted investigation should explore plants as medicinal tools (3).

Helminths are known to be a big problem in livestock production along the tropics. The nature of helminthic diseases is persistent and debilitating, and it is likely to result in morbidity and significant economic and community distress in both humans and animals. Weakness, anorexia, decreased food capability, weight loss and decreased productivity are the characteristic features of parasitic gastroenteritis, which was caused by mixed infection of various species of intestinal worms. As no effective anthelmintic vaccines were developed yet, chemotherapy remains the only treatment and effective tool to control and cure helminthic infections. The use of synthetic anthelmintics unsystematically can result in parasitic resistance. Herbal medicine has been in use to prevent development of parasitic resistance since ancient times (4). The importance of phytochemicals such as tannins, terpenoids, flavonoids, alkaloids and glycosides with anthelmintic activity was reported by other investigators (5, 6).

*Cayratia auriculata* belongs to native species of Southern India. *Cayratia auriculata* (Roxb.) gamble is a synonym of *Cyphostemma auriculatum* (Roxb), a climber that is well grown in dry evergreen to dry deciduous forests and well distributed in Andhra Pradesh (7, 8), Tamilnadu (9), Maharashtra (10), Madhya Pradesh (11), Orissa (12),

Gujarat, Goa, Karnataka, Kerala, Bihar, West Bengal, Bangladesh, Myanmar and Rajasthan (13). An ethnomedicinal survey on *Cayratia auriculata* shows that this plant is used as a folklore medicine and blood purifier, in cardiac disorders (7), intestinal worm diseases (11), earache, wound abscess, dog bite, rheumatism, purulent wounds, wound healing, tumors, cough, colds and hydrocele, but also as a tonic (15) and an astringent. This plant has been also used in veterinary medicine to treat animal bloody dysentery and diarrhea (16). Phytochemical studies of *Vitaceae* family species suggest the presence of alkaloids, flavonoids, saponins, steroids, terpenoids, stilbenoids and tannins (17).

Given that various phytoconstituents of medicinal plants are known for their anthelmintic properties and substantial literature review did not report anything about the anthelmintic activity of *Cayratia auriculata*, the present study has been carried out to perform a preliminary analysis on the phytoconstituents of this plant and to assess their anthelmintic properties (*in vitro*).

## MATERIALS AND METHODS

### Plant collection and authentication

The plant was collected in and around forest areas of Araku valley, Visakhapatnam district, Andhra Pradesh, and it was identified and authenticated by Associate Professor, Department of Botany, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India. The voucher herbarium specimen number 22228 was deposited in Andhra University Herbarium (AUH), Botany Department, Visakhapatnam.

### Extraction of plant material

Fresh plant material of *Cayratia auriculata* was washed under running tap water to ensure the absence of any foreign organic matter, solid debris, organisms, fungi, etc, and then dried in the shade for seven days. About 1200 g of shade dried plant material was grinded with the help of grinder to make powder and preserved in an air-tight container. Successive solvent extractions were then performed by using a Soxhlet apparatus. About 120 g of *Cayratia auriculata* powdered material was packed into a thimble and extraction continued until the solvent in the extractor siphon tube became colourless by using various solvents in the order of increasing polarity: hexane, chloroform,

ethyl acetate, and methanol, respectively. Whatman No. 1 filter paper (42) was used for extract filtration. The filtered extracts were concentrated by using a vacuum oven at 40<sup>o</sup> C and were preserved in a cool and dark place until further use (17, 18). Afterwards, extracts were subjected to a preliminary analysis of their phytoconstituents and *in vitro* evaluation of their anthelmintic properties.

**Phytoconstituent analysis**

Preliminary analysis of extracts was carried out to identify the presence of various phytoconstituents

by employing standard protocols (20, 21). The results were summarized in Table 1 after conducting the following chemical tests.

**Tests for alkaloids**

(a) *Dragendorff’s test*. By adding 1 mL of Dragendorff’s reagent to 2 mL of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

(b) *Mayer’s test*. Few drops of Mayer’s reagent were added to 1 mL of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids.

	Constituents	Tests	Inference			
			Methanol	Ethyl acetate	Chloroform	Hexane
1	Alkaloids	Dragendorff’s test	+	+	-	-
		Mayer’s test	+	+	-	-
		Hager’s test	+	+	-	-
2	Flavonoids	Alkaline reagent test	+++	-	-	-
		Shinod’s test	+++	-	-	-
3	Tannins and phenolic compounds	Ferric chloride test	+++	++	+	+
		Lead tetra acetic acid test	+++	++	+	+
4	Proteins	Biuret test	++	++	+	-
		Ninhydrin test	++	++	+	-
5	Carbohydrates	Molish test	+	+	+	+
		Benedict’s test	+	+	+	-
		Fehling’s test	+	+	+	-
6	Glycosides	Keller Killiani test	+	+	-	-
7	Saponins	-	+++	-	-	-
8	Triterpenoids	Horizon test	++	-	-	-
9	Steroids	Salkowski test	++	+	-	+
10	Starch	Iodine test	-	-	-	-

(-) = absence; (+) = traces; (++) = moderate; (+++) = abundance of phytoconstituents

**TABLE 1.** Preliminary phyto analysis of the methanol, ethyl acetate, chloroform and hexane extracts of *Cayratia auriculata*

(c) *Hager's test*. Two milliliters of extract were treated with few drops of Hager's reagent. A yellow precipitate was formed, indicating the presence of alkaloids.

### Tests for flavonoids

(a) *Alkaline reagent test*. Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

(b) *Shinod's test*. Ten drops of dilute HCL and a piece of magnesium were added to 1 mL of extract, the resulting deep pink colour indicating the presence of flavonoids.

### Test for phenolic compounds and tannins

(a) *Ferric chloride test*. Two milliliters of 5% neutral ferric chloride solution were added to 1 mL of extract, the dark blue colouring indicating the presence of phenolic compounds and tannins.

(b) *Lead tetra acetate test*. One milliliter of lead tetra acetate solution was treated with 0.5 mL of extract, precipitate formation indicating the presence of phenolic compounds and tannins.

### Tests for proteins

(a) *Biuret test*. Two drops of 3% copper sulphate and few drops of 10% sodium hydroxide were added to 1 mL of extract, violet or red colour formation indicating that proteins are present.

(b) *Ninhydrin test*. Two drops of 0.2% freshly prepared ninhydrin solution added to 1 mL of extract. Production of purple colour shows the presence of proteins.

### Test for carbohydrates

(a) *Molish test*. Few drops of alcoholic  $\alpha$ -naphthol solution were added to 2 mL of extract. Later, few drops of concentrated  $H_2SO_4$  were added along the walls of test tube. At the junction of two liquids, a violet colour ring appeared, indicating that carbohydrates were present.

(b) *Benedict's test*. To 5 mL of Benedict's reagent, 8-10 drops extract were added, then heated for five minutes; the resulting dark red precipitate indicated the presence of carbohydrates.

(c) *Fehling's test*. To 2 mL of extract, an equal volume of Fehling's (A & B) solution was added and heated for five minutes, the resulting red/dark

red precipitate indicating the presence of carbohydrates.

### Tests for glycosides

*Keller Killiani test*. A solution of 0.5 mL, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 mL of extract. Later, 1 mL of concentrated  $H_2SO_4$ , was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicated the presence of cardiac glycosides.

### Tests for saponins

A drop of  $Na_2CO_3$  solution was added to 5 mL of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins.

### Test for triterpenoids

*Horizon test*. Two milliliters of trichloroacetic acid was added to 1 mL of extract. The presence of terpenoids was confirmed by the formation of a red precipitate.

### Test for steroids

*Salkowski test*. The test extract was shaken with chloroform and concentrated  $H_2SO_4$  was added along the walls of a test tube; a red colour appeared, indicating the presence of steroids.

### Test for starch

*Iodine test*. Two milliliters of iodine solution with potassium iodine were added to 2 mL of test extract, and the appearance of a blue colour indicated that presence of starch.

### Anthelmintic property

Adult Indian earth worm, *Pheretima posthuma* (Annelida) was used to evaluate the anthelmintic property of different extracts of *Cayratia auriculata*, due to its anatomical and physiological similarity with round worm parasite occurring in the alimentary tract of human beings. The method of Ajaiyeoba *et al* and Kaur *et al* with slight modifications was used to evaluate anthelmintic activity (22, 23). An average size of 4-6 cm of adult earth worms were collected from water logged soil areas of GSL Medical College and General Hospital campus, and all faecal matters were removed by proper washing in normal saline. Extracts at concentrations of 20 mg/mL, 40 mg/mL and 80 mg/mL were used to identify the time of paralysis and

death of earth worms. Albendazole at a concentration of 10 mg/mL was taken as standard and 2% Tween 80 in distilled water was used as control. Freshly prepared test solutions and standard drug solutions were used for the entire experimental protocol. Six earth worms in each group of approximately similar size were placed into 10 mL of different test solutions. The time taken for the paralysis and death of individual earth worms was noted. No movement of worms, except when they were vigorously shaken, was noted as time of paralysis. No motility and fading of worm body color was deemed as death of worms. □

### RESULTS

#### Preliminary phytoconstituent analysis

Successive extraction of *Cayratia auriculata* was done in the order of increasing polarity of the solvent, and hexane > chloroform > ethyl acetate > methanol extracts were prepared. Qualitative preliminary phytoconstituents screening of

the various extracts of *Cayratia auriculata* showed the presence of various phytoconstituents. The methanolic extract was found to have a high content of flavonoids, saponins, tannins and phenolic compounds, moderate amounts of proteins, triterpenoids and steroids, and only traces of alkaloid carbohydrates and glycosides. The ethyl acetate extract was found to have moderate amounts of proteins, tannins and phenolic compounds, and traces of carbohydrates, glycosides, steroids, alkaloids. The chloroform extract had only traces of proteins, carbohydrates, tannins and phenolic compounds. The hexane extract had carbohydrate steroids, tannins and phenolic compounds. This shows that high polar phytoconstituents were extracted with methanol when compared to other solvents such as ethyl acetate, chloroform and hexane (Table 1).

#### Evaluation of anthelmintic property

Table 2 shows the mean paralysis and death time produced by the various extracts of *Cayratia auriculata* against adult earth worms. For the metha-

	Concentration (mg/mL)	Time taken for paralysis (min) Mean±SD	Time taken for death (min) Mean±SD
Control (2% Tween 80)	0	Nil	Nil
Standard (Albendazole)	10	26.73±0.56	87.70±0.91
Hexane extract	20	91.70±0.81	188.59±0.86
	40	65.03±0.66	151.18±0.61
	80	41.81±1.07	118.10±0.56
Chloroform extract	20	73.94±0.72	164.48±1.15
	40	55.06±1.04	131.00±1.25
	80	32.39±1.59	104.80±0.98
Ethyl acetate extract	20	61.92±0.93	141.00±1.04
	40	48.37±0.95	110.46±0.82
	80	29.09±1.05	97.26±0.66
Methanolic extract	20	52.38±0.79	124.66±0.97
	40	31.51±0.69	89.24±0.88
	80	21.98±0.88	79.06±0.87

SD=standard deviation

TABLE 2. Anthelmintic property of hexane, chloroform, ethyl acetate and methanolic extracts of *Cayratia auriculata*

nolic extract at a concentration of 80 mg/mL and albendazole at a concentration of 10 mg/mL, used as positive control, the mean paralysis time and death time were (P)21.98±0.88, (D)79.06±0.87 and (P)26.73±0.56, (D)87.70±0.91, respectively. The paralysis time and death time of all four extracts at 20 mg/mL was too long when compared to the standard drug and methanolic extract at a concentration of 40 mg/mL and 80 mg/mL. Chloroform and ethyl acetate extracts at 80 mg/mL showed a better paralysis time, but the death time was moderately long when compared to the standard drug. For the ethyl acetate extract at 80 mg/mL concentration, the paralysis time was closer to the standard drug but the death time was moderately high. All extracts expressed a dose dependent anthelmintic activity, with an increasing pattern in the following order: hexane, chloroform, ethyl acetate and methanol. The anthelmintic property of the *Cayratia auriculata* methanolic extract at a concentration of 80 mg/mL was found to be stronger than that of the standard albendazole at a concentration of 10 mg/mL. □

## DISCUSSION

Phytochemicals found in *Cayratia auriculata* during the preliminary phytoconstituent screening (Table 1) would be responsible for the evaluated anthelmintic activity (Table 2) and may optimistically deemed as a substitutive approach to control helminth infections (24, 25). All extracts of *Cayratia auriculata* exhibited concentration dependent anthelmintic properties on earthworms, probably due to the presence of alkaloids, glycosides, flavonoids, saponins, triterpenoids, tannins and phenolic compounds. Among all extracts, the methanolic one exhibited the most effective anthelmintic activity, most likely due to the involvement of glycosides, flavonoids, tannins and phenolic compounds; hence, it may be considered as a potent anthelmintic agent. The decreasing pattern of anthelmintic properties of the four different extracts was as follows: methanolic > ethyl acetate > chloroform > hexane extracts. At 40 mg/mL and 80 mg/mL, the methanolic extract of *Cayratia auriculata* exhibited a good anthelmintic property when compared to the standard at a concentration of 10 mg/mL. The methanolic extract of *Cayratia auriculata* expressed anthelmintic property in concentration dependent order with

shortened time of paralysis (P) and death (D) at 40 mg/mL and 80 mg/mL when compared to the ethyl acetate, chloroform, and hexane extracts at the same concentrations. The methanolic extract of *Cayratia auriculata* at 80 mg/mL caused paralysis and death of *Pheretima posthuma* at 21.98±0.88 and 79.06±0.87 minutes, respectively, while when the standard drug, albendazole, was used at concentration of 10 mg/mL, paralysis occurred at 26.73±0.56 minutes and death at 87.70±0.91 minutes. Uptake of glucose by the larval and adult stages of the susceptible parasites is impaired by albendazole, resulting in depletion of their glycogen stores. Helminths' metabolic pathways are disrupted by high concentrations of albendazole by inhibiting metabolic enzymes such as malate dehydrogenase and fumarate reductase, resulting in decreased energy produced by the Krebs cycle. Due to inadequate ATP production, the parasite is immobilized and ultimately dies (26).

Velebny GHS and Sirama *et al* reported that alkaloids exhibit neurotoxic properties by inhibiting acetylcholine esterase, resulting in acetylcholine induced body wall muscle spasms, flaccid paralysis and death of gastrointestinal parasites. On the other hand, plant glycosides at low concentrations, when ingested by human beings, kill intestinal parasites due to their neurotoxic properties (27, 28). Tannins are polyphenolic compounds with a highly effective action against internal nematodes in ruminants. Their great effectiveness as anthelmintic substances is thought to be related to their direct vermucidal activity. Tannins also play a role in increasing host resistance against intestinal parasites (29). Drugs such as oxiclosamide, bithinol and niclosamide are chemically belonging to synthetic phenolic compounds that exhibit their anthelmintic activity by uncoupling oxidative phosphorylation, which leads to a decreased ATP production, and as a result, the parasite is paralyzed and eventually dies. It was assumed that tannins present in the extracts produced similar results. Moreover, tannins bind to free proteins in the alimentary tract of host animals or glycoprotein in cuticle of the parasite produce anthelmintic activity (30). The current study showed that all extracts of *Cayratia auriculata* had an anthelmintic activity and the methanolic one could be a promising tool for an alternative approach to treat helminthic infections. □

## CONCLUSIONS

The present study aimed to perform a preliminary phytochemical analysis and evaluation of the anthelmintic activity (in-vitro) of various extracts of *Cayratia auriculata* (Family: Vitaceae) against earthworms (*Pheretima posthuma*). The phytochemical tests for various constituents revealed that the plant extracts contained alkaloids, flavonoids phenolic compounds, tannins, glyco-

sides, saponins, and steroids. Our findings highlighted that the methanolic extract of *Cayratia auriculata* had a significant anthelmintic activity. Further studies are needed to determine the actual phytoconstituents and underlying mechanisms responsible for the anthelmintic property. □

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