



# Preliminary Phytochemical Study and in Vitro Evaluation Anthelmintic Activity of Fresh Aerial Parts of *Centella Asiatica* L.

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## KEYWORDS

*Centella asiatica* L., *Pheretima Posthuma*, Preliminary phytochemical studies, flavonoids, Anthelmintic.

## ABSTRACT:

**Introduction:** Today among various infection that are afflicting human beings, Helminthes infections are the most prevalent type affecting a substantial proportion of the population that exist on earth.

**Objectives:** In recent years Medicinal plants are witnessing an upsurge in observations and documentation of traditional medicinal practices, as evidenced through a number of ethnomedicinal papers. Among various plants that show promising ethnomedicinal claims, *Centella asiatica* L. is one of those plants that have ethno medicinal claims against Helminthes. The present study is therefore aimed at validating the ethno medicinal claims.

**Methods:** The Preliminary phytochemical studies of the extract were carried out for Chloroform, ethanol and aqueous extract. Anthelmintic activity of ethanolic extract was carried out on Indian earthworm *Pheretima Posthuma* against the reference drug Albendazole.

**Results:** The phytochemical studies indicated the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides and starch in different extracts. Significant Anthelmintic properties were observed at higher concentration. The ethanolic extract of *Centella asiatica* L. caused paralysis in 5.29 min and death in 10.32 min. in 80 mg/ml concentration against the reference drug Albendazole at 40 mg/ml concentration has shown paralysis at 6.27 min and occurrence of death at 12.21 min.

**Conclusions:** The results suggest that there is an urgent need to isolate and evaluate the active constituents responsible for the exhibited biological activity.

## 1. Introduction

Helminthic infections, one of the most prevalent infections in humans, impacting a significant proportion of the global population. They are a major public health risk in developing nations, where they also increase the risk of pneumonia, eosinophilia, anaemia, malnutrition etc. Helminthes also affect millions of domestic animals and cause significant economic losses to domestic and outdoor animals. Due to the limited availability and affordability of modern medicines, people show dependency on traditional medicines to treat helminthic infections [1].

Among a large number of herbal plants that grow around us, *Centella asiatica* L. (Apiaceae) a small, herbaceous annual plant, native to warmer regions of both hemispheres like India, Sri Lanka, Bangladesh, Northern Australia, Indonesia, Iran, Malaysia, Melanesia, Papua New Guinea, and other parts of Asia [2-3]. In China, it is indigenously called as Gotu kola, and over 2000 years ago, it was one of the documented "Miracle elixirs of life". It is a small trailing herb which possesses glabrous stem, with pink striations and rooting at nodes [4]. It is one of popular plants having important ethnomedicinal claims for various medicinal uses like antimicrobial, bitter stomachic, cardi tonic, antidote against poison,



cuts and wound healing, memory enhancer, anti-inflammatory, anti-depressant, anti-fertility, antispasmodic activity etc. along with very potent anthelmintic activity [5-6].

It has a wide range of chemical substances which includes Betulic acid, Asiaticoside A & B, madecassoside, centelloside, Isothankunic acid, Isobrahmic acid, Oleic acid, Linoleic acid, Linolenic acid [7-8]. It is widely used in Ayurvedic, African and Chinese system of medicine. The present study aims to validate the ethnomedicinal claim for Anthelmintic properties of fresh aerial part of *Centella asiatica* L. [9-10].

## 2. Objectives

To validate the ethnomedicinal claims of fresh aerial parts of the plant *Centella asiatica* L.

## 3. Methods

**Plant Materials:** The fresh aerial part of the plant *Centella asiatica* L. was collected from Nadia Nursery, Chakdaha, Nadia, West Bengal during the month of June 2023 and authenticated by Dr. Suchandra Samanta Mandal, M.Sc. (Botany), KU, M Phil. (Education), KU, Assistant Professor, K. Bed College, Krishnanagar, West Bengal, India.

**Chemicals and Reagents:** The chemicals, solvents and reagents used in the study were of standard analytical grade obtained from S. D Fine Chem Ltd., Mumbai and Loba Cheme, Mumbai.

**Animals:** Adult Indian earth worm- *Pheretima posthuma* of about 5-7 cm long and 0.3-0.4 cm in width were used for this study.

**Standard drug:** Albendazole 400 mg Tablet (GlaxoSmithKline) was used as standard.

**Preparation of extract:** The plant material was dried thoroughly in shade condition at room temperature. It was then subjected to size reduction process. 250 gm. of the fresh aerial part of the plant *Centella asiatica* L. was defatted with petroleum ether. The marc was thoroughly dried and subjected to cold maceration process with different solvent (12 hrs. each) like chloroform, ethanol and water. The extracts obtained were dried at

temperature below 40°C to obtain concentrate of the crude extract [11-12].

**Phytochemical Screening:** Phytochemical screening was carried out using standard procedures [13]. Different qualitative tests were performed on the crude extracts to identify the various active constituents that are present in the fresh aerial part of the plant *Centella asiatica* L. The results are depicted in **Table 1**.

**Determination of anthelmintic activity:** The Anthelmintic activity was investigated on mature Indian earthworm *Pheretima posthuma*, which shares morphological and physiological similarities with human intestinal roundworm parasites. The Anthelmintic method was carried as per the method of Pal et al. with minor modifications [14]. Sixteen groups each containing six earthworms of approximately equal size were released into 10 ml of desired formulation. Each group was treated with, Albendazole, chloroform extract, ethanol extract and aqueous extract (40, 60 and 80 mg/ml) in normal saline with 5% DMF and in vehicle alone acting as control. Time for paralysis was noted when no movement could be observed with a slight pin prick method. Time for death of individual earth worms was recorded when the worms showed no movement either by vigorous shaking or by dipping in warm water.

## 4. Results

**Qualitative Chemical Tests of the Extracts:** Preliminary phytochemical screening of different extracts indicated the presence of alkaloids, flavonoids, carbohydrates, tannins, and other compounds (**Table 1**).

**Anthelmintic Activity:** The result of Anthelmintic Activity is given in **Table 2** and result plotted in **Figure 1**. The result of Anthelmintic activity on earthworm *Pheretima Posthuma* revealed that the ethanolic extract at different concentration has shown paralysis effect and has caused death of earthworms. Significant Anthelmintic properties were observed in a dose dependent manner. The mean  $\pm$  SEM values [statistical analysis] were calculated for the extract. The ethanolic extract of *Centella asiatica* L. caused paralysis in 5.29 min and death in 10.32 min. in 80 mg/ml concentration. The reference drug Albendazole at 40 mg/ml concentration has shown paralysis at 6.27 min and occurrence of death at 12.21 min.

**Table 1:** Qualitative chemical tests of the extracts

Test		Chloroform Extract	Ethanollic Extract	Aqueous Extract
Alkaloids		+	+	-
Amino Acids	Millon's Test	+	+	+
	Ninhydrine Test	+	-	+
Carbohydrates	Molisch's Test	-	-	+
Flavonoids	Alkaline reagent Test	+	+	+
	Zinc hydrochloride Test	+	+	-
Glycosides	General Tests	+	+	+
	Borntrager's Test	+	+	+
	Modified anthraquinones Test	-	-	-
	Baljet's Test	+	+	+
	Legal's Test	+	+	+
	Froth formation Test	-	-	-
Mucilage		-	-	-
Tannins	Chlorogenic acid Test	-	+	+
	Ferric Chloride Test	-	+	+
	Gelatin Test	-	+	+
Proteins	Xanthoproteic Test	-	-	-
Starch		+	+	+
Steroids	Liebermann - Burchard Test	+	+	-
	Salkowski Test	+	+	-

[“+” = Present and “-” = Absent]

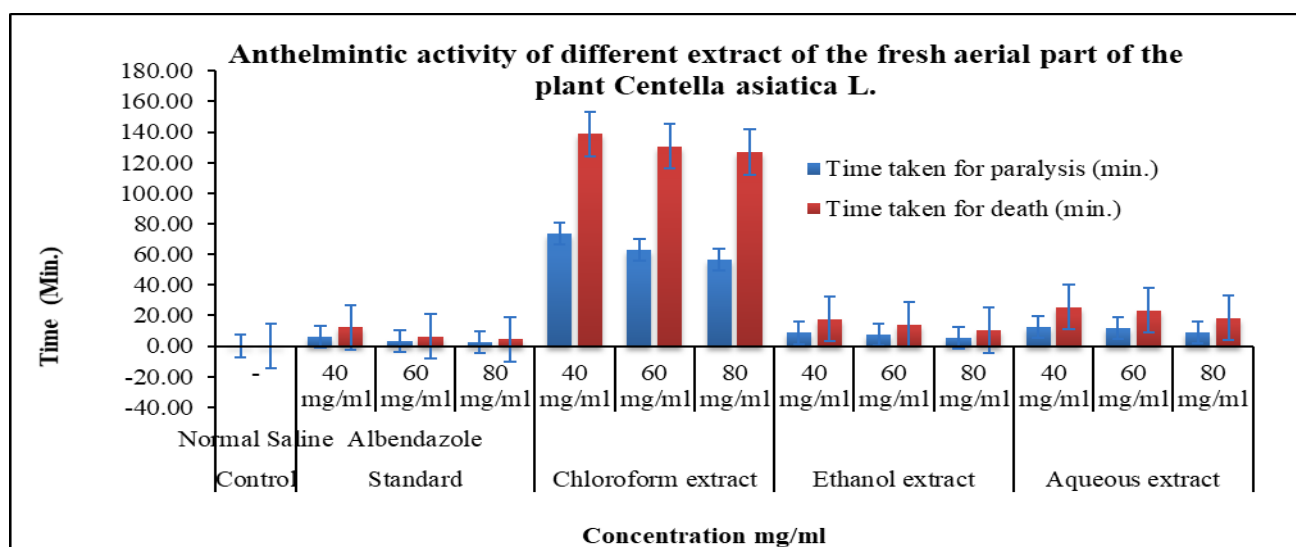
**Table 2:** Anthelmintic activity of different extract of the fresh aerial part of the plant *Centella asiatica* L.

Group Treatment Dose			Reaction time in (minutes)	
			Time taken for paralysis (P)	Time taken for death (D)
Control	Normal Saline	-	0.00	0.00
Standard	Albendazole	40 mg/ml	6.27±0.05	12.21±0.03
		60 mg/ml	3.16±0.21	6.39±0.02
		80 mg/ml	2.34±0.03	4.46±0.04
Chloroform extract		40 mg/ml	73.32±0.09	138.73±0.22
		60 mg/ml	63.16±0.04	130.55±0.06
		80 mg/ml	56.31±0.27	126.86±0.05



Ethanol extract	40 mg/ml	8.58±1.15	17.54±0.04
	60 mg/ml	7.38±0.05	14.08±0.01
	80 mg/ml	5.29±0.06	10.32±0.16
Aqueous extract	40 mg/ml	12.32±0.03	25.38±1.22
	60 mg/ml	11.45±0.05	23.16±0.21
	80 mg/ml	8.75±0.27	18.31±0.06

Results are expressed as mean  $\pm$  SEM of three observations. P – Paralysis; D – Death mg: milligram, ml: millilitre



**Figure1:** Values of paralysis and death time of *Pheretima posthuma* [Indian adult earth worms] plotted against varying concentration of different extract of fresh aerial part of the plant *Centella asiatica* compared with standard (Albendazole).

## 5. Conclusion:

Natural product compounds derived from medicinal plants and their counterparts have provided a number of clinically relevant drugs. Natural source compounds have gained in relevance in recent years due to enormous chemical diversity they supply. According to Ayurvedic, African and Chinese system of medicine, *Centella asiatica* is a very potent & ethno medicinally important plant. Literature review reveals that *Centella asiatica* L. has pharmacologically & medicinally essential compounds like Asiaticoside A & B, madecassoside, centelloside, Isothankunic acid etc. The present study reveals the presence of various phytochemical constituents in aqueous, chloroform & alcoholic extract of the leaves. The study has also revealed prominent Anthelmintic activity comparable to that of standard drug Albendazole suggesting a need to isolate and evaluate the active constituents responsible for the exhibited biological activity.

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