



PHYTO CHEMICAL AND ANTHELMINTIC EVALUATION OF CORM OF *AMORPHOPHALLUS CAMPANULATUS*

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ABSTRACT

The present study deals with phytochemical and anthelmintic evaluation of corm of *Amorphophallus campanulatus*. This evaluation revealed the presence of many phytochemical constituents. Corm was extracted with petroleum ether, chloroform and methanol. Crude tannins were isolated from methanol extract. All extracts and crude tannins were evaluated for anthelmintic activity. Chloroform, methanol extracts and crude tannins showed very good anthelmintic activity. Paralysis and death times of crude tannins were very close to the standard drug Albendazole.

KEY WORDS

Amorphophallus campanulatus, elephant foot yam, crude tannins and anthelmintic activity.

INTRODUCTION

Amorphophallus campanulatus (Dennst) belonging to the family of Araceae is commonly known as elephant foot yam. It is distributed throughout India. *Amorphophallus campanulatus* is a stout herbaceous plant with underground hemispherical depressed dark brown corm. The corms are acrid, astringent, thermogenic, irritant, anodyne, anti-inflammatory, antihemorrhoidal,

expectorant, carminative, digestive, appetizer, stomachic, anthelmintic, liver tonic and aphrodisiac¹. Corm contains triacontane, lupeol, betulinic acid, stigmaterol, β - sitosterol and its palmitate, glucose, galactose, rhamnose and xylose². Amblyone, a triterpenoid isolated from *Amorphophallus campanulatus* showed good antibacterial activity³. Methanolic extracts of tuber showed significant analgesic activity in mice⁴. Salviasperanal a triterpenoid isolated from



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Amorphophallus campanulatus showed antibacterial activity⁵. Ethanolic extract of tuber have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats⁶. A water soluble polysaccharide isolated from the aqueous extracts of the corm was found to contain d- galactose, d- glucose, 4-o-acyl-d-methyl galacturonate and 1- arabinose⁷. Corm showed tyrosinase and laccase activity⁸. Tubers showed antiprotease activity⁹. 3, 5- diacetyl tambulin, a flavanoids isolated from tuber showed good antibacterial activity¹⁰. The corm part was used traditionally for the treatment of helminthes but not yet proved scientifically. Hence, the present study was designed to evaluate the anthelmintic activity of petroleum ether, chloroform, methanoilc extracts and crude tannins obtained from corm part of the *Amorphophallus campanulatus*.

MATERIALS AND METHODS

(i) *Plant material:*

Corm of *Amorphophallus campanulatus* was collected in March 2009 from local market of Nalgonda, Andhra Pradesh, India. *Amorphophallus campanulatus* (Dennst) (Araceae) was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Corm was cleaned, dried at room temperature and stored properly in air tight container. The dried material was then subjected to size reduction to obtain coarse powder using grinder. This powdered material of mesh # 16 was then used for further process.

(ii) *Preparation of different corm extracts:*

The powdered corm (2 kg) was extracted with solvents of increased polarity such as, petroleum ether, chloroform and methanol for 24 h with each solvent by hot extraction using Soxhlet apparatus at 60 °C. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a dessicator until used for further studies.

(iii) *Isolation of crude tannins:*

1 g of methanolic extract of corm was agitated vigorously with 80% acetone at 55 °C in a reactor fitted with stirrer and baffles to produce uniform mixing. The slurry after cooling was filtered and acetone was recovered. The left over slurry was suspended in distilled water (three times) and stirred to make it homogenous. It was filtered and dried¹¹.

(iv) *Percentage purity of isolated crude tannins:*

100 mg of isolated crude tannins was dissolved in 5 ml of water and filtered. From the filtrate 1 ml was pipetted out and diluted to 7.5 ml with water. To this 0.1 ml indigo sulphonic acid solution was added and titrated against 0.01N potassium permanganate solution until the color changes to golden yellow. Percentage of total tannins was calculated as per the standard procedure¹¹.

(v) *Phytochemical study:*

Powder material was subjected to fluorescence and chemical analysis. A portion of residue from each extract and crude tannins was



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subjected to phytochemical analysis in order to know the presence of steroids, alkaloids, tannins, proteins, glycosides, carbohydrates, phenols, flavonoids, volatile oils, saponins and starch^{12,13}.

(vi) *Thin layer chromatography:*

All the extracts of corm were subjected to thin layer chromatography to determine the number of spots and corresponding R_f values by developing in different solvent systems. TLC was performed using pre-coated silica gel TLC plates of E-Merck, Germany. The developed TLC plates were observed under daylight, UV light, iodine chamber and by spraying various detecting agents.

(vii) *Anthelmintic activity:*

Prepared extracts and isolated crude tannins were evaluated for anthelmintic activity separately. Adult Indian earth worms, *Pheretima posthuma* were chosen for the study due to their anatomical and physiological resemblance with the intestinal round worm parasite of human beings¹⁴. They were collected from Nalgonda region and identified by Sri Prasad Traders, Nalgonda, Andhra Pradesh, India. The earthworms of nearly equal size around 6 cm were acclimatized to the laboratory condition before experimentation. The earth worms were divided into six groups of six earth worms in each. Albendazole was diluted with 5% DMF (Dimethyl Formamide) in normal saline solution to obtain 10, 25 and 50 mg per ml served as standard and poured into petri dishes. The extracts were dissolved in 5% DMF in normal saline solution and diluted to prepare three

concentrations such as 10, 25 and 50 mg per ml and poured into petri dishes. 5% DMF in normal saline solution was taken as control. Earth worms were placed in petri dishes containing different concentrations of standard and extracts as well as crude tannins at room temperature. The mean paralysis time and mean death time for each sample was calculated (all the readings were taken in triplicate). The time taken for worms to become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates and induces movement in earth worm if alive¹⁵.

RESULTS AND DISCUSSION:

1. *Extraction:*

Corm of *Amorphophallus campanulatus* was extracted with petroleum ether, chloroform and methanol. % yield of petroleum ether, chloroform and methanol extracts were found to be 0.47 (yellow color mass), 0.54 (brown color mass) and 2.56 (reddish brown color mass) respectively. % purity of tannins in chloroform and methanol extracts was found to be 0.0052 ± 0.03 and 0.038 ± 0.35 respectively (n=3). Amount of tannins in methanolic extract was greater than chloroform extract. Therefore, crude tannins were isolated from methanolic extract and the yield was 0.002%. The % purity of tannins in crude tannins was found to be 0.042 ± 0.03 (n=3).

2. *Phytochemical analysis:*

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Table 1
Fluorescence analysis of corm of Amorphophallus campanulatus

Treatment	Visible Light	Ultra violet Light
Powder as such	Cream	Cream
Powder + 5% Sulphuric acid	Reddish Brown	Black
Powder + Ethanol	Cream	Brown
Powder + 1N Sodium hydroxide	Brown	Brown

The powdered drug showed color change under visible light and ultraviolet light after treatment with different chemical reagents. This fluorescence analysis revealed the presence of chemical constituents with fluorescent character. The results were given in Table.1. Treatment of powdered drug with different chemical reagents had revealed the presence of different chemical constituents. The results were given in Table.2.

Table 2
Chemical analysis of corm of Amorphophallus campanulatus

Treatment	Observation
Powder as such	Cream
Powder + 2% Ferric chloride	Light green
Powder + 10% Sodium hydroxide	Dark Brown
Powder + Sodium hydroxide + Water	Dark Brown
Powder + 5% Potassium hydroxide	Dark Brown
Powder + Water, shake	Foam formation
Powder + Ethanol	Cream
Powder + Sulphuric acid	Dark Brown
Powder + Hydrochloric acid	Light Brownish Cream
Powder + Nitric acid	Dark Brown

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Preliminary phytochemical screening revealed the presence of various phytochemical given in the following table.

Table 3*Phytochemical screening of corm of Amorphophallus campanulatus*

Constituents	Petroleum ether extract	Chloroform extract	Methanolic extract
Steroids	+	-	-
Alkaloids	-	+	+
Tannins	-	+	+
Glycosides	-	+	+
Carbohydrates	-	-	+
Phenols	-	+	+
Flavonoids	-	-	+
Volatile oil	-	-	-
Saponins	-	-	+
Proteins	-	-	+
Starch	-	-	+

“+” indicates the presence of constituents “-” indicates the absence of constituents

Petroleum ether extract showed the presence of steroids. Alkaloids, tannins, glycosides and phenols were found in chloroform extract. Alkaloids, tannins, proteins, glycosides, carbohydrates, phenols, flavonoids, saponins and starch were found in methanolic extract. Crude tannins isolated from methanol which were subjected to phytochemical analysis showed positive result for tannins, phenols and flavonoids but negative for alkaloids, proteins, glycosides, carbohydrates and saponins indicating that crude tannins had some amount of phenol and flavonoids.



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3. TLC analysis:

Table 4
TLC analysis corm of *Amorphophallus campanulatus*

Extracts	Solvent system	R _f values
Petroleum ether	Benzene: Ethyl acetate [9:1]	0.62;0.50;0.87
Chloroform	Toluene: Ethyl acetate: Diethyl amine[7:3:1]	0.18;0.21
Methanol	Benzene: Ethylacetate [9:1]	0.28;0.59;0.71;0.88

Thin layer chromatography was performed for all the extracts and results were shown in Table. 4. Crude tannins showed more than one spot on TLC plates. This may be due to the presence of phenols and flavonoids along with tannins.

4. Evaluation of anthelmintic activity:

Table 5
Anthelmintic activity of corm of *Amorphophallus campanulatus*

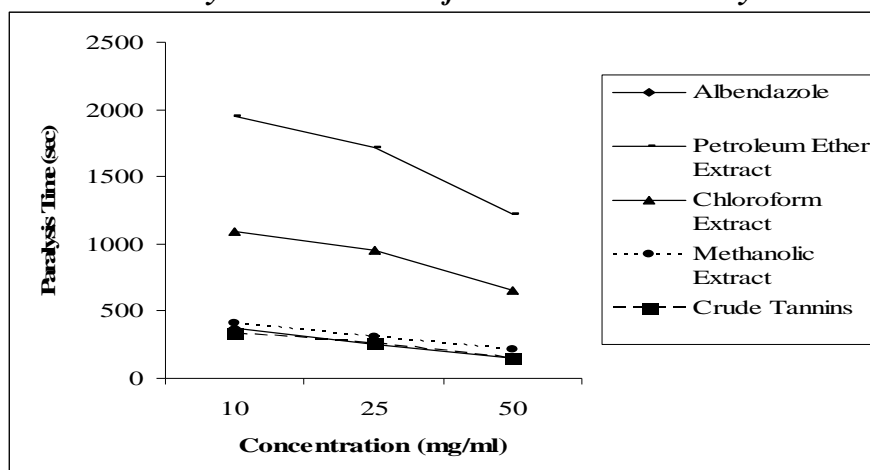
Extract	Concentration (mg/ml)	Time in minutes (Mean \pm SEM where n=3)	
		For paralysis	For death
Control (5% Dimethyl formamide in Saline solution)		-----	-----
Standard Albendazole	10	6:16 \pm 0.01	7:08 \pm 0.03
	25	4:15 \pm 0.01	5:30 \pm 0.01
	50	2:28 \pm 0.01	3:37 \pm 0.014
Petroleum ether	10	32:50 \pm 0.05	35:46 \pm 0.06
	25	28:40 \pm 0.02	30:49 \pm 0.01
	50	20:20 \pm 0.02	23:22 \pm 0.23
Chloroform	10	18:13 \pm 0.05	20:02 \pm 0.05
	25	15:49 \pm 0.04	17:18 \pm 0.01
	50	10:54 \pm 0.01	12:19 \pm 0.32
Methanol	10	6:52 \pm 0.02	8:29 \pm 0.05
	25	5:04 \pm 0.02	7:31 \pm 0.01
	50	3:30 \pm 0.03	6:15 \pm 0.03
Crude tannins	10	5:34 \pm 0.04	7:24 \pm 0.01
	25	4:20 \pm 0.03	6:36 \pm 0.06
	50	2:31 \pm 0.02	3:40 \pm 0.05



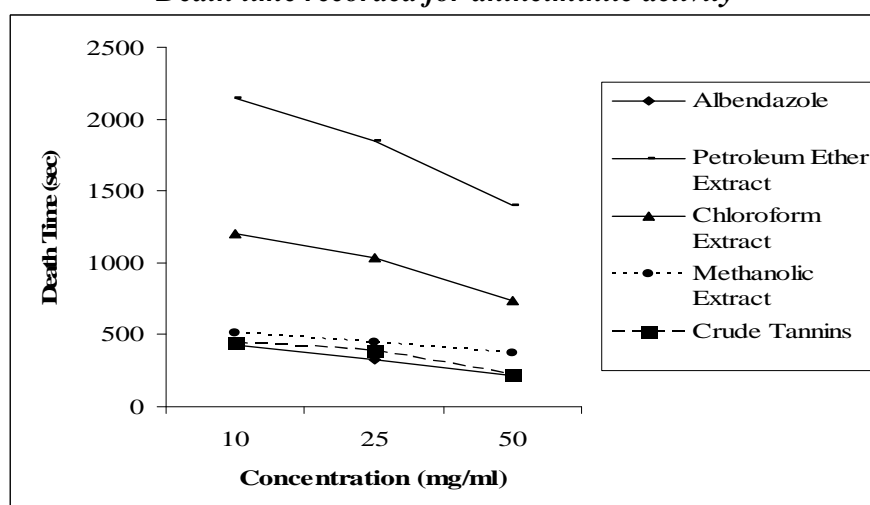
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Petroleum ether, chloroform, methanol extracts were evaluated for anthelmintic activity and isolated crude tannins. The results of paralysis and death time were shown in the above table and also in graphs.

Graph 1
Paralysis time recorded for anthelmintic activity



Graph 2
Death time recorded for anthelmintic activity





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Among the extracts, methanolic extract showed very good anthelmintic activity. Methanolic extract had paralysed the earthworms within 6.52 min, 5.04 min and 3.30 min and caused death within 8.29 min, 7.31 min and 6.15 min at the concentrations of 10, 25 and 50 mg per ml respectively. Control was observed for more than 8 hrs and no paralysis and death were occurred. Crude tannins isolated from methanolic extract were evaluated for anthelmintic activity at various concentrations in mg per ml level. At all the concentrations, paralysis and death time of crude tannins were less than all the extracts. Paralysis and death times of crude tannins were very close to paralysis and death times of Albendazole.

CONCLUSION:

The present study on phytochemical analysis confirms the presence of different phytochemicals in corm of *Amorphophallus campanulatus*. The values of paralysis time and death time of methanolic extract as well as isolated crude tannins were very close to the values of albendazole. So the corm of *Amorphophallus campanulatus* possesses anthelmintic activity. Future plan of work includes purification and characterization of isolated tannins from corm of *Amorphophallus campanulatus*.

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