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Pharmacognostical and Anthelmintic Activity of *Tephrosia calophylla Bedd.*

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Abstract

Aim: To investigate Pharmacognostical and Anthelmintic activity of various extracts of *Tephrosia calophylla* Bedd. tubers and leaves against *Pheretima posthuma.* **Methods:** In pharmocognostic studies different types of evaluations were carried out that focus on macroscopic, organoleptic, physicochemical, extractive values and phytochemical screening. The different extracts of *T. callophyla* tubers and leaves were investigated for anthelmintic activity using earth warms. Two different concentrations (5 and 10 mg/ml) of each extract were tested in the assay, which involved the determination of paralysis time and death time of the organisms. *Albendazole* is used as standard. **Results and Conclusions**: *T. callophyla* tubers and leaves crude drugs for macroscopic / organoleptic are carried out to check purity, quality and standardization of crude drugs. Physicochemical analysis of powdered drugs extractive values and ash values were estimated to know the drug detecting adulteration, qualitative and quantitative profiles of crude drugs constituents is well understood. Phytochemical screening revealed that flavonoids phenols and alkaloids are rich source in most extracts. The results showed that methanol extract at 5 mg/ml possesses strong vermicidal activity and found to be effective as an anthelmintic.

Keywords
Tephorosia callophyla, Pharmacognostical studies, Anthelmintic activity, *Pheretima posthuma* Leaves and Tubers

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INTRODUCTION

Parasitism, especially by helminthic species, impairs health by causing lack of diarrhea, appetite, anemia and, in severe cases, death (1). Synthetic anthelmintic have been used throughout world for decades to minimize the losses caused by helminthic infection. However, parasite resistance increases costs, reduces production efficiency along with the risk of contamination of the animal products (2-4) and increases the risk of environmental contamination (5). Advancement of Anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants has an alternative source of anthelmintics. Herbal drugs have been in use since ancient times for the treatment of parasitic disease in human and could be of value in preventing the development of resistance (6).

The genus *Tephrosia* is a rich contribution to promising biological activities. *Tephorosia callophyla* is perennial under shrub found widely in Talakona forest of Andhra Pradesh, South india (7). According to Ayurveda the plant is useful as an

antianthelmintic, antiulcer, antihepato protective, anti-pyretic, antileprosy, antidiu and as well as an alexiteric drug (8). *T.callophyla* have revealed the isolation of 23 different compounds of which 18 were known and are new.

MATERIALS AND METHODS

Plant material:

The tubers and leaves material of *Tephorosia callophyla* were collected during September - December 2018 from Talakona forest in Tirupati, Andhra Pradesh, India. The taxonomic identification of the plant is confirmed by Prof. N. Yasodamma. The voucher specimen BKTC:3 were deposited in the herbarium, (RUK) Department of Botany, Rayalaseema University, Kurnool for future reference as per standard methods (9). The present work was carried out in the Department of Botany, Rayalaseema University, Kurnool. Plant materials were thoroughly washed and then dried under shade for one week. The dried parts were ground in a mixer grinder and sieved. The powders were stored in air sealed polythene bags at room temperature until further use.

PHARMACOGNOSTIC EVALUATION

Macroscopic / morphological and organoleptic characters:

Habit, morphology; colour, odour, taste, texture, size and shape of tubers and leaves were assessed (10-11).

Physicochemical analysis:

Determination of ash values:

Ash values such as total ash, acid insoluble ash, water soluble ash, sulphated ash and moisture content/loose of weight on drying, values were determined with the powders of tubers and leaves (12).

Total Ash:

1g of air-dried powders was taken separately in a previously ignited and weighed silica crucible. The powder was spreaded in an even layer and ignited by gradually increasing the heat up to 500 -600^{0}C until it becomes white, indicating the absence of carbon. Then crucible was cooled in desiccator. The ash was weighed and percentage of total ash with reference to air dried powder was calculated.

$$\%\,\text{of Total Ash Value} = \frac{\text{Weight of the Ash}}{\text{Weight of the crude drug taken}} \times 100$$

Acid insoluble ash:

25ml of hydrochloric acid (70 g/l) was added to the crucible containing the total ash and boiled gently for 5 minutes. The insoluble matter was collected on the ash less filter paper and washed with hot water until the filtrate is neutral. The filter paper was transferred to the original crucible and ignited to a constant weight. The residue was allowed to cool in a suitable dessicator for 30 min. The ash was weighed without delay and percentage of acid-insoluble ash with reference to air dried powder was calculated.

$$\%\,\text{of Acid insolu ble Ash} = \frac{\text{Weight of the acid insolu ble ash}}{\text{Weight of the crude drug taken}} \times 100$$

Water soluble ash:

25 ml of water was added to the crucible containing the total ash and boiled for 5 min. The insoluble matter was collected on the ash less filter paper and washed with hot water. The filter paper was transferred to the original crucible and ignited to a constant weight at a temperature not exceeding 450^{0}C. The residue was allowed to cool in a suitable desiccator for 30 minutes. The weight of the residue was subtracted from the weight of total ash. The ash was weighed without delay and percentage of water soluble ash with reference to air dried powder was calculated.

$$\%\,\text{of Water Solube Ash Value} = \frac{\text{Weight of the Total Ash-Weight of the water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

Sulphated ash:

A silica crucible was heated to red for 10 minutes and was allowed to cool in a desiccator and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until white fumes are no longer evolved and ignited at 800^{0}C ± 25^{0}C until all black particles have disappeared. The ignition was conducted in a protected place from air currents. The crucible was allowed to cool. A few drops of concentrated sulphuric acid was added and heated. Ignited as before and was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5 mg.

$$\% \text{ of Sulphated Ash Value} = \frac{\text{Weight of the Sulphated Ash}}{\text{Weight of the crude drug taken}} \times 100$$

Moisture content / Loss on drying:

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter can be driven off under specified conditions. 2 g of dried powder of tubers and leaves were accurately weighed and placed in a previously dried weighing bottle. The sample was heated at 100 - 105^{0}C until two consecutive weighing does not differ by more than 5 mg. The loss of weight in mg material was calculated.

$$\% \text{ of Moisture Content} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample taken}} \times 100$$

Extractive value determination:

Fifty grams of coarsely powdered air-dried material of tubers and leaf were macerated with 250 ml of each solvents, placed in a glass stoppered conical flask (Aqueous, Acetone, Alcohol, Benzene, Chloroform, Ethyl acetate, Methanol and Petroleum ether) shaking frequently, and then allowing it to stand for 18 hrs. Filter it rapidly through what man No.1 filter paper, taking care not to lose any solvent. Transfer 25 ml of filtrate to flat- bottom dish and evaporate the solvent on a water bath. Dry at 105^{0}C for 6 hours, cool in a desiccator for 30 minutes and weigh it immediately. Calculate the content of extractable matter in % of air-dried material (13).

$$\text{Extraction yield}(\%) = \frac{W_1}{W_2} \times 100$$

Where W_1 = Net weight of the extract in gm after extraction.
W_2 = Weight of the total powder taken in gms.

Phytochemical analysis:

Preliminary Photochemical Screening:

To detect the different classes of secondary metabolites in the crude extracts of tubers and leaves of *T. callophyla* preliminary phytochemical analysis was undertaken by adopting standard qualitative methods (14).

Preparation of crude drug extracts:

Dried tubers and leaves powder (50 g in 250 ml) were extracted with Aqueous, Acetone, Alcohol, Benzene, Chloroform, Ethyl acetate, Methanol and Petroleum ether. The drug was soaked for 72 hrs. and the filtered extract was dried on water bath stored at 4^{0}C in refrigerator.

Preparation of test solutions:

The preliminary tests for the detection of secondary metabolites was carried out for all the extracts (Methanol, ethanol, ethyl acetate, chloroform, benzene, acetone petroleum ether and aqueous) of tubers and leaves. 500 mg of each extract was dissolved in 100 ml of the respective solvent and filtered through Whatman filter paper No.1. Thus the filtrate obtained was used as test solution for the following preliminary phytochemical screening tests.

ANTHELMINTIC ACTIVITY

Worm collection:

The earthworms *Pheretima posthuma* of approximately equal size (8 cm) was collected from Yamuna vermi compost, Peddatekuru, Kurnool Dist., A.P.

Reference Drug:

Albendazole: It was prepared by dissolving in distilled water at the concentrations of 5mg and 10 mg.

Preparation of Desired Formulation:

Desired Formulation can be prepared by dissolving the standard concentrations of 5 and 10 (Aqueous, Acetone, Alcohol, Benzene, Chloroform, Ethyl acetate, Methanol and Petroleum ether) in 25 ml of Distilled Water.

Experimental procedure:

The Aqueous, Acetone, Alcohol, Benzene, Chloroform, Ethyl acetate, Methanol and Petroleum ether extracts of tubers and leaves were investigated for their anthelmintic activity against *P. posthuma*. Various concentrations (5 and 10 mg) of each extract were tested in the bioassay, which involved the determination of time of paralysis and time of death of the worms. *Albendazole* was included as standard reference and distilled water as control. The anthelmintic assay was carried as per the standard method (15). Worms collected and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworms of 8cm in length and 0.5-0.8 cm in width were used for all the experimental protocol. Fifteen groups of approximately equal sized earthworms consisting of two in each group were released into 25ml of desired

formulation. Three groups were prepared as control distilled water, warm water, Reference drug *Albendazole* (5 mg and 10mg) and remaining as drug extracts Aqueous, Acetone, Alcohol, Benzene, Chloroform, Ethyl acetate, Methanol and Petroleum ether (5mg and 10mg). Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body color.

Statistical analysis:

All the data are expressed as mean ± SEM. The data obtained from the various groups were statistically analyzed using one way ANOVA followed by Dunnett's test. These values $p^{*}<0.05$ (significant); $p^{**}<0.01$ (more significant); $p^{ns}>0.05$ (not significant) were considered to indicate a significant difference between the groups.

RESULTS AND DISCUSSION

ABOUT THE SELECTED MEDICINAL PLANT

Common names:

Telugu: Adavivempalli, Dumpavempalli, Gaddavempalli, kommuvempalli.

Taxonomic classification:

Kingdom: Plantae
Division: Magnoliophyta
Class: Dicotyledons
Sub class: Polypetaceae
Series: Polypetaceae
Order: Rosales
Family: Fabaceae
Genus: *Tephrosia*
Species: *Calophylla* Bedd.

Distribution:

Tephrosia calophylla is a perennial under shrub found widely in Andhra Pradesh, South India. It is mainly available in localities of hill slopes, rare in shady locations. It is found widely in Talakona forest of Andhra Pradesh, India.

Description: (Plate: 1)

T. calophylla is a perennial under shrub which exhibits greater diversity. Roots are Rhizomatous (or) tuberous Leaves are simple, coriacius, oblanceolate, entire, mucronate, parallel, petiolate, winged, anticulate at apex. Flowers are Light pink, glabrous, composed mucronate, interminalrecemes plants start flowering in April to August every year.

Medicinal uses:

The root is diuretic, allays thirst, enriches blood, cures diarrhea, and is useful in bronchitis, inflammations, antidiabetic, boils and pimples. Leaves are tonic to intestines, and a promising appetizer. The seeds can be used as substitute for coffee. This plant contains a wide variety of flavanoids and isoflavanoids. Due to the presence of these compounds it exhibits several medicinal properties such as antihelmintic, anti-pyretic and an alexiteric drug. It is also active against leprosy, ulcers, and used as alternative cures for diseases of the liver, spleen, heart and blood.

PHARMACOGNOSTIC EVALUATION

Table-1: Macroscopic / Organoleptic Studies

S. No	Parameters	Tubers	Leaves
1	Color	Green	Brown
2	Shape	Long scarlet	Coriaceous
3	Size (cm)	35-40cm × 10-12cm	7-10cm x 2-3cm
4	Texture	Slightly Rough	Smooth
5	Taste	Bitter	Bitter
6	Odour	Characteristic	Characteristic

Organoleptic Studies (Plate-1; Table-1):

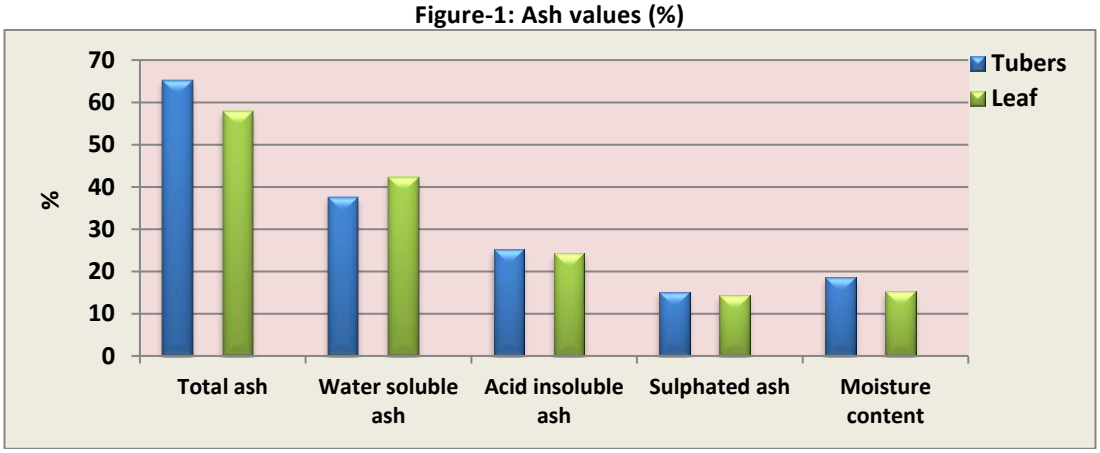
Color: Tuber powder Brown: Leaf green: **Odour**: Both are characteristic: **Taste**: Both are bitter: **Texture:** Tubers slightly rough: Leaves Smooth: **Shape: Tubers** Long Scarlet: Leaves Coriaceous. Morphological studies and physiochemical constants help in the standardization of the crude drugs. Study of Organoleptic characteristics provides firsthand information about the quality of raw material used for the study.

Physicochemical analysis:

Table-2: Ash values (%)

Plant parts	Total ash	Water soluble ash	Acid insoluble ash	Sulphated ash	Moisture content
Tubers	65.15	37.60	25.15	15.15	18.50
Leaf	57.95	42.20	24.30	14.30	15.25

Figure-1: Ash values (%)

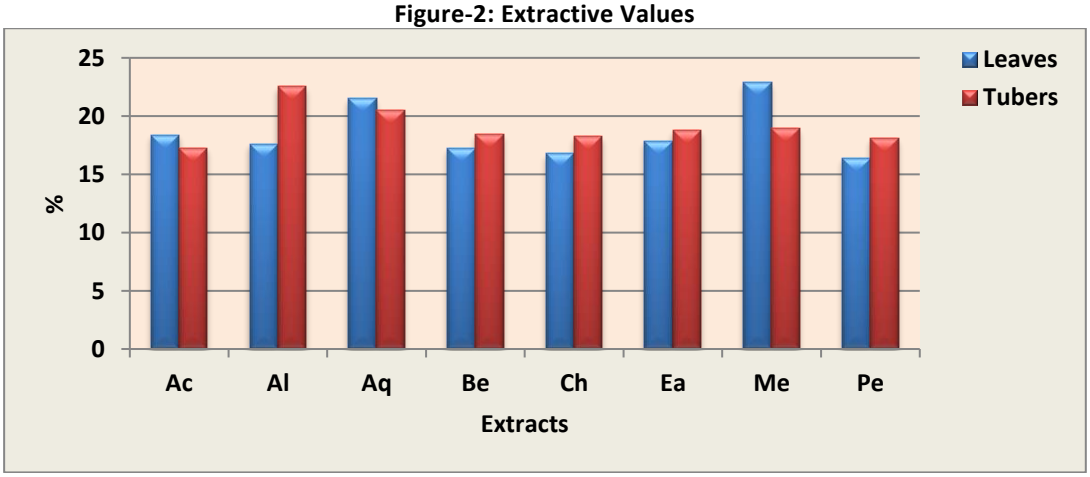


Powdered drug Ash values (%): (Table-2; Figure-1)
Total ash 65.15**%, water soluble ash** 37.60%, **acid insoluble ash** 25.15% **sulphated ash 15.15%** and **Moisture content** 18.50% were more in tubers than leaves. Ash values of any crude drug gives an idea about the presence of earthy matter and /or inorganic composition and /or other impurities present along with the crude drug.

Table-3: Extractive Values (%w/w)

Extracts	Extraction		Filtrate color		Extract nature and color	
	Leaves	Tubers	Leaves	Tubers	Leaves	Tubers
Ac	18.40	17.25	Blackish green	Darkorange	Smooth& Blackish green	Rough&Darkorange
Al	17.60	22.55	Blackish green	Light orange	Solid&Green	Sticky& Light orange
Aq	21.52	20.50	Green	Light yellow	Powdery& Light yellow	Sticky& Light orange
Be	17.20	18.45	Light green	Light ornge	Smooth& Light green	Sticky& Light ornge
Ch	16.80	18.25	Blackish green	Darkorange	Rough& Blackish green	Smooth&Darkorange
Ea	17.86	18.75	Green	Light ornge	Powdery &Green	Powdery& Light ornge
Me	22.88	18.95	Light green	Orange	Smooth & Light green	Sticky solid& Orange
Pe	16.40	18.05	Light green	Light yellow	Smooth& Light green	Rough&light brown

Figure-2: Extractive Values



Extractive Values (Table-3, Figure-2)
Extractive values in leaves yielded highest amount in methanol and aqueous22.88w/w, 21.52w/w, lowest in petroleum ether16.40w/w respectively. Filtrate color of leaves powders exhibit blackish green to light green. Tubers yielded highest amount in aqueous 22.55w/w, lowest in acetone 17.25w/w. Filtrate color of tubers powders exhibit dark orange to light yellow. Extractive values represented the presence of compounds in polar and non-polar

solvents. It is useful for the diversity of chemical nature and property of drug contents.

Table-04: Preliminary phytochemical screening

TEST	Leaves								Tubers							
	AC	AQ	AL	BE	CH	EA	ME	PE	AC	AQ	AL	BE	CH	EA	ME	PE
Alkaloids																
Mayers	+	++	-	+	++	-	-	+	++	+	+	++	++	+	+	-
Wagner's	++	+	-	+	-	-	-	-	++	-	-	++	++	+	++	+
Flavonoids																
Shin dons	++	++	-	-	-	-	++	-	-	++	-	-	-	-	++	-
$FeCl_3$	-	+	-	-	-	-	-	+	++	++	-	+	+	++	++	-
Phenols																
$FeCl_3$	++	++	++	++	++	-	++	++	++	+	++	-	++	+	-	-
Ellagic acid	-	-	-	-	++	-	-	-	-	-	++	++	-	-	-	-
Glycosides																
Keller –Kilani	++	++	+	+	-	-	+	+	++	++	++	++	-	++	++	++
Tannins																
$FeCl_3$	+	+	-	-	++	-	-	-	++	++	-	-	-	-	-	-
Steroids																
Salkowski	+	+	-	++	++	-	++	-	-	+	++	-	++	++	++	++
Quinones	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Lignins																
Labat test	++	-	++	+	+	-	-	-	-	-	-	-	-	-	-	-
Saponins	++	-	++	++	-	-	++	++	-	+	-	-	++	-	-	++

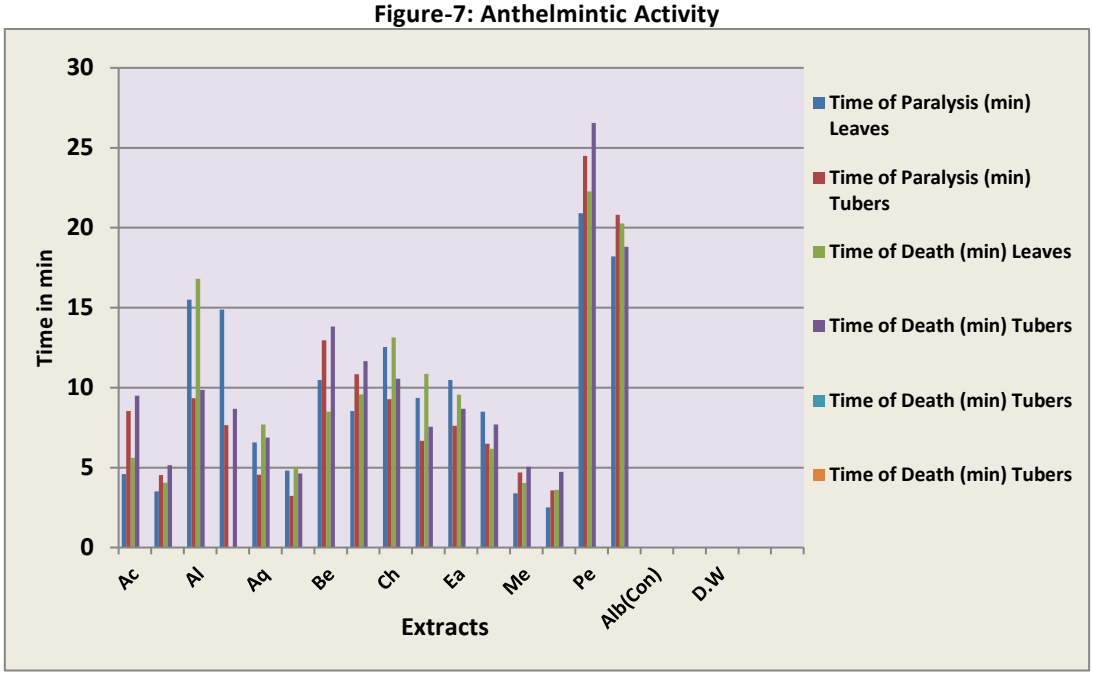
"++" indicates -Abundant presence; **"+" indicates** -(Slightly presence); **"– "indicates** - Absent
AC: Acetone, **AL:** Alcohol, **AQ:** Aqueous, **BE:** Benzene, **CH:** Chloroform, **EA:** Ethyl acetate, **ME:** Methanol, **PE:** Petroleum ether

Preliminary Phytochemical screening (Table-4)
Tubers and leaves both yielded highest amounts and more number of secondary metabolites are alkaloids, phenols, flavonoids tannins, saponins and glycosides: followed by leaves consists low quantities of phyto-constituents as steroids; tubers as tannins. Benzene extract of tubers shows without quinones. Lignins are totally absent in tubers.

Table-9: Anthelmintic activity-Time for Paralysis and Death of the Worm (min)

Extracts	Con. In mg	Time of Paralysis (min)		Time of Death (min)	
		Leaves	Tubers	Leaves	Tubers
Ac	5	4.60±0.24**	8.5±0.28**	5.62±0.20**	9.5±0.24**
	10	3.52±0.36**	4.54±0.37**	4.06±0.30**	5.16±0.24**
Al	5	15.50±0.28**	9.34±0.26**	16.80±0.24**	9.86±0.20**
	10	14.88±0.28**	7.66±0.24**	15..18±0.73*	8.68±0.20**
Aq	5	6.58±0.32**	4.55±0.36**	7.69±0.47**	6.87±0.30**
	10	4.8±0.16**	3.22±0.36**	5.05±0.60**	4.64±0.37**
Be	5	10.4±0.36**	12.96±0.35**	8.50±0.28**	13.83±0.12**
	10	8.54±0.28**	10.84±0.12**	9.58±0.24**	11.66±0.24**
Ch	5	12.54±0.37**	9.28±0.24**	13.14±0.24**	10.56±0.24**
	10	9.36±0.26**	6.67±0.21**	10.86±0.20**	7.56±0.24**
Ea	5	10.46±0.36**	7.62±0.24**	9.56±0.24**	8.68±0.20**
	10	8.50±0.28**	6.50±0.36**	6.18±0.24**	7.69±0.47**
Me	5	3.40±0.24**	4.6±0.21**	4.04±0.36**	5.06±0.30**
	10	2.50±0.14**	3.5±0.38**	3.62±0.25**	4.74±0.37**
Pe	5	20.90±0.20*	24.5±0.28**	22.28±0.16*	26.55±0.28**
	10	18.20±0.36**	20.8±0.28**	20.26±0.20*	18.8±0.28**
Alb(Con)	5	80.92±0.84		86.10±0.14	
	10	52.10±0.12		60.20±0.16	
D.W	15ml	-		-	

Figure-7: Anthelmintic Activity



Anthelmintic Activity (Table-9, Figure-7)

Aqueous, alcohol, acetone, benzene, chloroform, ethyl acetate, methanol and petroleum ether leaves and tubers extracts of *T. calophylla*were against anthelmintic activity with 5 and 10 mg / 25 ml of distilled water against earth worms and compared with the *Albendazole* the standard anthelmintic drug at 5 and 10 mg/25 ml distilled water observed for the paralysis and death of worms. The results revealed that methanol extracts are more effective followed by acetone and aqueous extracts and all the extracts showed better activity than the standard drug in taking less time for the paralysis and death of the worms. As the results indicated that time for paralysis 80.92; 52.10; Death 86.10; 60.20 minutes with *Albendazole*.

CONCLUSION

Morphological studies of the leaves and tubers give the standard values for future studies. Physicochemical analysis resulted in the highest water soluble ash content which helps in the drug designing. Moisture content is very less which proved long life and storage of the crude drug. Leaves and tubers methanol extract at the doses of 5 and 10mg can be suggested as the effective drug against Anthelmintic activity without causing any toxicity. All extracts shows effective paralysis and death than standard drug albendazole. It would be interesting to identify the active principle responsible for the anthelmintic activity and to study its further pharmacological actions.

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