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ANTI TUBERULAR AND ANTHELMINTHIC ACTIVITIES OF AQUEOUS METHANOLIC EXTRACT OF *CARALLUAMA ATTENUATA*

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Keywords:

Caralluma attenuata, Cold Maceration, Anti Tubercular Activity, Micro Plate Alamar Blue Assay Method, Anthelminthic Activity and Piperazine Citrate

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ABSTRACT: *Caralluma attenuata* is a perennial small, leafless succulent plant which grows in tropical and subtropical areas. It belongs to the family *Apocyanaceae*. The folkal information reveals that the tribes generally chew the whole plant to treat peptic ulcer, diabetes, in weight reduction to suppress hunger and also to produce endurance. It has been reported for the presence of steroids, flavanoids, saponins, triterpenes and pregnane glycosides etc., several biological activities like anti-diabetic and anti ulcerogenic were reported. In the present study an attempt is made to evaluate Anti tubercular and Anthelminthic activities for aqueous methanolic (50:50) extract after carrying out the phytochemical investigations. Anti tubercular activity was evaluated by Micro plate Alamar blue assay method against streptomycin, ciprofloxacin and pyrazinamide as standard drugs. Anthelminthic activity was evaluated on *Perithima posthuma* using Piperazine citrate as standard drug. The results obtained for biological activities were shown to posses considerable Anti tubercular activity at 50 µg/ml and Anthelminthic activity at 300mg/ml.

INTRODUCTION: Tuberculosis is the second major global infectious disease. As per WHO survey.8.7 million cases were reported in 2012 and 1.4 million people died. In low income and middle income countries over 95%of TB deaths were occurred due to malnutrition which leads to impaired immunity. It is one of important reason for prognosis of TB infection.

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Intestinal parasitic infections are the most common form of public health problem even in the developed countries. Chronic helminthiasis causes severe anaemia which leads to death because of vital organ infections. According to WHO survey the children of age group 7-11 years are more prone to the helminth infections because of poor sanitation. However, some of the foods which contain bitter glycosides, tannins etc; act as natural Anthelminthics.

Since the literature survey revealed that tribal have been chewed the stem parts of *Caralluma attenuate* [2] as the best remedy to cure several diseases like obesity [3-5], diabetes [6-7], athereosclerosis [8] etc because of their medicinal properties, the

researcher aimed to evaluate Anti tubercular and Anthelminthic activities for aqueous methanolic extract of whole stem part of *Caralluma attenuata* belongs to family *Apocynaceae*[1]. The researcher has used advanced methods to evaluate the activities. The methods and results were explained as following.

MATERIALS AND METHODS:

Collection of plant material:

The whole stem parts of *Caralluma attenuata* was collected in Ananthapuramu district, Andhra Pradesh. The plant was authenticated by Taxonomist Professor. J. Sreeramulu, Sri Krishna Devaraya University, Anantapuramu.

Preparation of extract:

The dried and powdered stem part of *Caralluma attenuata* was passed through a sieve no.22 and each kilo gram was extracted successively by cold maceration [9] with 2.5 litres of aqueous methanol (50:50). The extract was concentrated to dryness under reduced pressure using rotary vacuum evaporator. The extract was subjected for the phytochemical investigations [10]. The identified chemical constituents were reported in **Table 1**. The extract was utilised to evaluate Anti tubercular and Anthelminthic activities. The results obtained were tabulated in **Table 2** and **3** respectively.

Anti tubercular activity:

The aqueous methanolic extract was dissolved in chloroform (RP1), ethanol (RP2) and ethyl acetate (RP3) then these solutions was subjected for anti TB activity.

The Antitubercular activity [11-14] of extract was assessed against *M. tuberculosis* using Micro plate Alamar Blue Assay (MABA)[15].This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionised water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on plate. The final drug concentrations tested was 100 to 0.2 µg/ml. Plates was covered and sealed with Para film and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink as shown in the **Fig. 1** and **Fig. 2.**

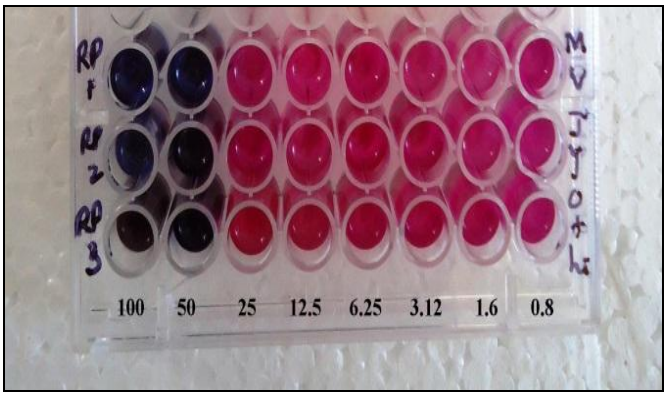

FIG.1: ANTI TB ACTIVITY OF SAMPLES

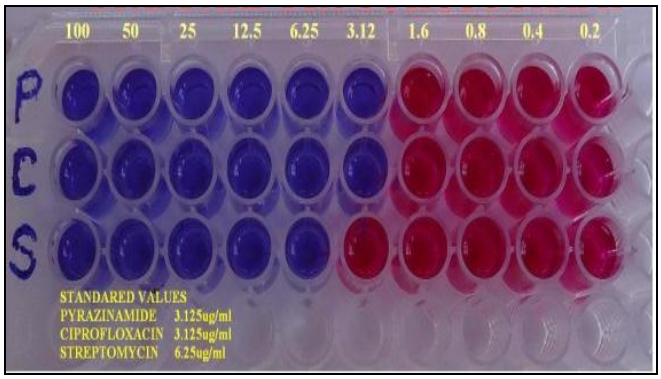

FIG. 2: ANTI TB ACTIVITY OF STANDARD DRUGS

Anthelminthic activity:

The Anthelminthic activity [16-18] was carried out using adult earthworms (*pheretima posthuma),* belongs to the group of Annelida. 20ml of aqueous methanolic(50:50) extract of *caralluma attenuata* (test solutions) containing three different concentrations (100mg/ml, 200mg/ml, and 300mg/ml in distilled water) were prepared and taken in three different petridishes and 5 earthworms were placed in each petridish. 20 ml of Piperazine citrate [19] (10mg/ml concentration) was used as reference standard while distilled water was used as the control. Time taken for paralysis of worms was noted for the sample and standard solution; where there was no movement of any sort could be observed except the worms was shaken

vigorously. Time of death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50degrees centigrade. The observations were explained in results **Table 3.**

RESULTS AND DISUSSION:

TABLE 1: PHYTOCHEMICAL STUDIES

Compound	Name of chemical test	Result
Alkaloids	Dragandroffs test	Positive
	Mayer's test	Positive
	Wagner's test	Positive
	Hager's test	Positive
	Muroxide test	Negative
Cardiac glycosides	Killer killani test	Positive
Anthraquinone glycosides	Borntragers test	Positive
Cyanogenetic glycosides	Guignaurd reagent test	Negative
	Guaiacum resin and copper sulphate test	Negative
Caumarin glycosides	Aromatic odour test	Negative
	Filter paper test	Negative
Tannins and phenolic compounds	5%$FeCl_3$ solution	Positive
	Dil. HNO_3	Positive

Anti tubercular activity:

The Micro plate alamar blue assay method was used for evaluation of Anti tubercular activity. The Anti tubercular activity was Sobserved at 50 µg/ml solution for all the three solvents.

TABLE 2: ANTI TUBERCULAR ACTIVITY

S.no	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	RP-1	S	S	R	R	R	R	R	R
2.	RP-2	S	S	R	R	R	R	R	R
3.	RP-3	S	S	R	R	R	R	R	R

S: Sensitive R: Resistance

Anthlemintic activity:

The results obtained for Anthelminthic activity was represented as follows.

TABLE 3: ANTHELMINTHIC ACTIVITY USING AQUEOUS METHANOLIC EXTRACT

Groups	Concentration(mg/ml)	Earth worms	
		Paralysis time	Death time
Plant extract	100mg/ml	38min	45min
(Aqueous methanolic extract)	200mg/ml	10min	19.45 min
	300mg/ml	8min	16.20min
Distilled water (control)	—	—	—
Piperazine citrate	10mg/ml	25 min	64min

The aqueous methanolic extract of *Caralluama attenuata* showed significant Anthelminthic activity at all concentrations, but the maximum activity was observed at 300mg/ml.

DISCUSSION: The aqueous methanolic extract was found to posses the considerable Anti tubercular and Anthelminthic activities. Hence the researcher has evaluated the activities by *in vitro* methods; the present study becomes one of the positive evidence to explore the research for further investigations.

CONCLUSION: The aqueous methanolic extract of *Caralluma attenuata* may be considered as preventive natural herb for helminthiasis since it has shown the better anthelminthic activity within 16 minutes than the standard drug. Further the principle active constituents which are responsible for antitubercular activity can be isolated. The mechanism for both of these activities would be

explained by carrying out the in-vivo methods in future research.

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