Research Article

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PRELIMINARY PHYTOCHEMICAL AND ANTHELMINTIC SCREENING OF LEAVES, BARK, ROOTS OF PLANT *TABERNAEMONTANA CORONARIA*

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ABSTRACT
Tabernaemontana coronaria is a potential non cultivated medicinal plant widely distributed in waste lands and open forests in India. The alcoholic, ethereal and chloroform extracts were obtained from the plant *Tabernaemontana coronaria* by soxhlet extraction or continuous hot percolation method and the extracts were evaluated for their anthelmintic activity, against locally available earth worms (*Pheretima posthuma*). Three concentrations (10, 30, 60 mg/ml) were prepared from each extract and were used for the study over earth worms. The study involves the determination of time of paralysis and time for death of the earth worms tested. The results obtained from the study revealed that all the extracts of *Tabernaemontana coronaria* possess significant anthelmintic effect at highest concentration (60 mg/ml). A solution of Albendazole was prepared in same concentration as that of extract and was used as standard. A 5% tween-80 suspension prepared using normal saline solution was used as control or blank.
Keywords: *Tabernaemontana coronaria, Pheretima posthuma*, anthelmintic activity, 5% tween-80 suspension, normal saline.

INTRODUCTION

Helminthiasis[1] is an infection caused by parasitic worms such as roundworms and pinworms. The worms usually infect only the intestinal tract but sometimes they may invade other organs. The type and severity of symptoms is determined by the type of worm and the part of the body infected. Infected[2] people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation. Infestation can cause morbidity, and sometimes death, by compromising nutritional status, affecting cognitive processes, inducing tissue reactions, such as glaucoma, and provoking intestinal obstruction or rectal prolapse. Helminthic therapy, a type of immunotherapy, is the treatment of autoimmune diseases and immune disorders by means of deliberate infestation with a helminth or with the ova of a helminth. The gastrointestinal helminthes become resistant to currently available anthelmintic drugs therefore there is an increasing demand towards natural anthelmintics.

Tabernaemontana coronaria (syn. *Ervatamia coronaria*) is a glabrous, evergreen, dichotomously branched shrub, belonging to the family Apocynaceae. It is distributed in upper Gangetic plain, Garhwal, East Bengal, Assam, Karnataka, Kerala, and in Burma[3]. The root is acrid, digestible, with a bitter and bad taste. In Ayurveda, it is used in the treatment of kapha, biliousness, and diseases of the blood. It is aphrodisiac; tonic, especially to the brain, liver, and spleen. It is used as a purgative. It kills intestinal worms[10], and its root, when chewed, relieves toothache[11]. When rubbed with water and applied to wounds it prevents inflammation[3]. A number of chemical constituents including indole alkaloids, phenols, and sterols from the leaves, stems, and roots of the plant have previously been reported[5, 6]. Inflammation is elicited by numerous stimuli such as infectious agents, environmental factors, ischemia, antigen-antibody reaction, physiological and pathological factors, and also free radicals. Antioxidants thus play an important role to protect human body against damage by reactive oxygen species[7]. The anthelmintic activity of plant *T. coronaria* leaves was done by Latha[14] et al. The present study was undertaken to screen the anthelmintic activity of the leaves, bark and roots of *T. coronaria.*

MATERIAL AND METHODS

Plant material

Leaves, bark and root of plant *Tabernaemontana coronaria* were collected from the local areas of Nuzvid in the month of August. The voucher specimen was identified with Mr. M. Raghu Ram, Botanist in Acharya Nagarjuna University. Leaves, bark and root of *Tabernaemontana coronaria* were shade dried and it was milled into a coarse powder by a mechanical grinder and it was stored in a closed vessel for further use.

Preliminary phytochemical screening

The test reagent used for preliminary phytochemical screening was as follows[12, 13]

Alkaloids (Dragendorff,s reagent)

Alkaloids give reddish brown precipitate with Dragendorff,s reagent (potassium bismuth iodide solution).

Amino acids (Ninhydrin test)

Amino acids give violet color solution when boiled with ninhydrin solution.

Carbohydrates (Molisch's test)

Addition of few drops of alcoholic α-naphthol and few drops of concentrated sulphuric acid to the test solution through sides of test tube, results in appearance of purple to violet colour ring at the junction of two layers.

Tannins (Ferric chloride test)
The extract when treated with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.
Proteins (Trichloroacetic acid test)
A precipitate is formed when trichloroacetic acid is added to the test solution.
Steroids (Libermann-burchard test)
The extract when treated with few drops of acetic anhydride, boiled, cooled and then added with concentrated sulphuric acid from the sides of the test tube, a brown ring is formed at the junction of two layers and upper layer turns green which shows presence of steroids.
Flavonoids (Shinoda test)
A few magnesium turnings and concentrated hydrochloride acid was added drop wise, to the test solution. A pink, scarlet, crimson red or occasionally green to blue color appears after few minutes.
Saponins
Extract was triturated with small quantity of water and then filtered. To filtrate, distilled water was added up to 20 ml and shaken in a graduated cylinder for 15 min. One centimeter layer of foam indicates the presence of saponins.
Glycosides
200 mg of drug is extracted with 5 ml of dilute sulphuric acid by warming on water bath. It was filtered and then neutralized the with 5% solution of sodium hydroxide. 0.1 ml of Fehling's solution A and B was added until it becomes alkaline (test with pH paper) and is heated on a water bath for 2 minutes. The formation of red precipitate indicates the presence of glycosides.
Acids
To extracts little water was added and then filtered. To the filterate few ml saturated solution of sodium bicarbonate was added. Effervescence was observed which suggests the presence of acids
Phytosterols
One ml of concentrated sulphuric acid was added to extracts filtrate dissolved in 1ml of chloroform. A reddish brown colour exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of Phytosterols.
Triterpenoids (Salkowski test)
The extract is treated with few drops of concentrated sulphuric acid, a yellow colored lower layer indicates presence of Triterpenoids.
Phenols
Few mg of substance was dissolved in alcohol and treated with alcoholic ferric chloride the violet colour was observed suggests presence of phenols.
Preparation of extracts
The dried powder of about 60g is placed in soxhlet extractor and is extracted with 500ml (each) of ethanol, chloroform and ether solvents respectively for 48hrs. The process was again repeated with the same quantity of dried powder. The extract obtained was filtered and evaporated to dryness under reduced pressure in a rotary vacuum evaporator. The details of the plant extraction *Tabernaemontana coronaria* were presented in the table1 and also phyto-chemical constituents in the extract were presented in the table 2.
Anthelmintic assay
Chloroform, ether and alcohol extracts from the plant of *Tabernaemontana coronaria* were investigated for anthelmintic activity against *Pheretima posthuma*. Various concentrations (10, 30 and 60 mg/ml) were prepared using 5% tween80 suspension in normal saline and were tested. The bioassay involves determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference, normal saline as positive control and 5% tween 80 in normal saline as negative control. The Anthelmintic assay was carried as per the method followed by Venkata raju *et al* with minor modifications[8]. The assay was performed on adult Indian earth worms, *Pheretima prosthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings[9]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *In vitro*. The earthworms were collected from moist soil and washed with normal saline to remove all faecal matter and were used for the anthelmintic study. The earth worms of 8-10 cm in length and 0.3-0.4 cm in width were used for all experimental procedures. The earth worms were divided into fourteen groups containing six earth worms in each group. All the extracts and standard drug solution were freshly prepared with 5% tween80 in normal saline as suspension before starting the experiment. Different extracts and standard drug solutions were poured into different petri plates. All the earth worms were released into 1ml, 3ml and 6ml suspension which contain 10mg/ml of formulation as follows: chloroform extract, ether extract, alcohol extract and albendazole in three different concentrations. Observations were made for the time taken to paralysis and for the death of 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 when dipped in warm water (50ºC) followed with fading away of their body colors. The results were calculated by two way ANOVA.

Statistical Analysis
The results were compared using one way analysis of variance (ANOVA) followed by Dunnet's tests. *P*-values less than 0.05 were considered as indicative of significance.

Table 1: Details of the extractions of *Tabernaemontana coronaria* plant

	Solvent used	Color and Consistency	% yield
Plant material used for the total extraction 60g	Chloroform(CETC)	Greenish brown	2.64
	Ether (EETC)	Blackish brown	2.43
	Alcohol(AETC)	Greenish brown	2.78

Table 2: Phyto chemical constituents of various extracts of *Tabernaemontana coronaria*

Chemical Test	Chloroform Extract	Ether Extract	Alcohol Extract
Alkaloids	+	-	+
Amino acids	+	+	+
Carbohydrates	+	+	+
Tannins	+	-	+
Proteins	+	-	+
Steroids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Glycosides	+	-	+
Acids	+	-	+
Phytosterols	+	-	+
Triterpenoids	+	-	+
Phenols	+	+	+

Table 3: Anthelmintic activity of various extracts of plant *Tabernaemontana coronaria*

Treatment	Concentration used (mg/ml)	Time taken for Paralysis (min)	Time Taken for Death (min)
Control	Positive	-	-
	Negative	-	-
Albendazole	10	3.16±0.030	32.24±0.348
	30	3.18±0.054	30.69±0.146
	60	2.69±0.115	21.58±0.226
Chloroform	10	5.73±0.034	117.75±0.069
	30	4.70±0.023	85.43±0.086
	60	4.38±0.045	75.76±0.103
Ether	10	5.44±0.048	118.88±0.081
	30	4.61±0.037	89.75±0.101
	60	3.43±0.049	87.86±0.066
Alcohol	10	4.84±0.057	106.24±0.091
	30	4.53±0.073	84.55±0.095
	60	3.39±0.127	78.25±0.070

Values are mean ± SEM (n-6) one way ANOVA followed by Dunnet's test where * represents significant at < 0.05, ** represents highly significant at p < 0.01, *** represents very significant at p< 0.001.

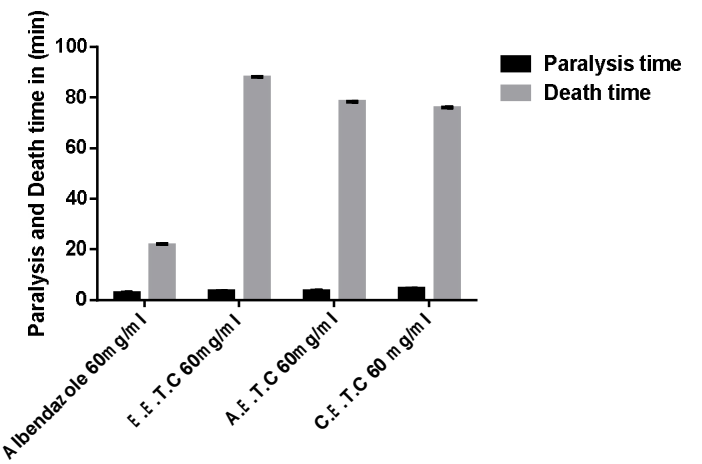

Figure 1: Anthelmintic activity of various extracts of *Tabernaemontana coronaria*

RESULTS AND DISCUSSION

The qualitative phytochemical investigation of the chloroform and alcoholic extracts of leaves, stem and roots of plant of *Tabernaemontana coronaria* showed the presence of active chemical constituents such as Alkaloids, Amino acids, Carbohydrates, Tannins, Proteins, Steroids, Flavonoids, Saponins, Glycosides, Acids, Phytosterols, Triterpinoids and Phenols. In the ether extract of the same, phytochemicals such as Tannins, Proteins, Glycosides, Acids, Phytosterols and Triterpinoids were absent. (Table 2).

The results of anthelmintic activity revealed that chloroform, ether and alcoholic extracts of leaves, stem and roots of plant of *Tabernaemontana coronaria* exhibit varying degree of activity like paralysis of worms followed by its death at all tested concentrations. From the above observations, the extracts of *Tabernaemontana coronaria* plant were found to show potential anthelmintic activity when compared to standard drug in a dose dependent manner (Table 3). Alcoholic extract of *Tabernaemontana coronaria* at a concentration of 60mg/ml showed paralysis at 03.39 min and death of earthworm at 78.25 min which was comparable to

standard Albendazole (Table 3). From the result, it is clear that the three extracts of *Tabernaemontana coronaria* plant have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug.

CONCLUSION

From the results it is evident that, the three extracts of *Tabernaemontana coronaria* leaves, stem and roots of plant demonstrate to possess dose dependant anthelmintic activity when compared to Albendazole (Figure 1). The anthelmintic activity of *Tabernaemontana coronaria* plant was found to be inversely proportional to the time taken for paralysis and time of death of the worms. The active constituents responsible for anthelmintic activity are present in all three extracts of *Tabernaemontana coronaria*. The possible mechanism of the anthelminthic activity cannot be explained on the basis of our present results. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for its anthelmintic activity.

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