

IN-VITRO ANTHELMINTIC ACTIVITY OF LEAF EXTRACTS OF *SHOREA TUMBUGGAIA ROXB.* AND *HOLOSTEMMA ADA KODIEN SCHULT.* ON (*PHERETIMA POSTHUMA*) INDIAN EARTHWORM.

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ABSTRACT

Objective: To evaluate anthelmintic activity for the leaf extracts of two folk medicinal plants from the southern Eastern Ghats of India; *Shorea tumbuggaia Roxb.* (Dipterocarpaceae) and *Holostemma ada Kodienschult* (Asclepiadaceae) to document the scientific evidence on folklore uses for worm infections.

Method: The methanolic and hydroalcoholic (*S. tumbuggaia Roxb.*) extracts and methanolic and ethyl acetate (*H. ada K. Schult.*) extracts were used for the evaluation of *in-vitro* anthelmintic studies using *Pheretima posthuma* (Indian earth worm), for the various concentrations ranging from 50, 100, 200 and 400 µg/ml for both extracts were tested in the bioassay involved determination of time of paralysis (P) and time of death (D) of the earthworm. Albendazole was used as standard reference and distilled water as control.

Results: The phytochemical investigation reveals the presence of tannins, flavonoids, saponins, terpenoids, steroids, anthocyanins glycoside and reducing sugars were the major phytoconstituents present in the extracts. ME-STR, EAE-HAKS and ME-HAKS (17.467 ± 1.525, 15.450 ± 1.981 and 12.267 ± 1.707) extract showed potent activity. All results were statistically analysed by using student 't' - test one-way ANOVA; the P < (0.0001) were significant when compared with standard group.

Conclusion: The paralytic and death rate of methanolic extracts of STR and HAKS and ethyl acetate extract of HAKS were found to exhibit anthelmintic activity. Further studies to be conducted for the isolation of bioactive compounds responsible for the anthelmintic activity.

Keywords: *Shorea tumbuggaia Roxb.*, *Holostemma ada Kodienschult*, Albendazole, *Pheretima posthuma*, anthelmintic.

INTRODUCTION

Helminthiasis is an infection through one or more intestinal parasitic larvae (roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), or hookworms (*Necator americanus* and *Ancylostoma duodenale*). Helminthiasis is one of the chief complications of livestock manufacture throughout the world, predominantly in tropical and subtropical zones. The ailment is particularly predominant in developing countries in association with poor organization, poor nutrition and insufficient regulatory measures. Influx of these infections can cause morbidity, and occasionally death, by bargaining nutritional status, affecting cognitive processes, persuading tissue responses, such as granuloma, and provoking intestinal obstruction or rectal prolapse. Control of helminthiasis is based on drug treatment, biological control, grazing management, vaccination, ethnoveterinary treatment, amended hygiene and health education reported by WHO (Dhar, *et al*, 1987).

Shorea tumbuggaia Roxb. is a tree taxon, IUCN Red List of Threatened Species, belonging to the family Dipterocarpaceae; *S. tumbuggaia Roxb.* with economic and medicinal values (Patil K.S. *et al*, 2004). The genus *Shorea* (family Dipterocarpaceae) is native to Southeast Asia, from northern India to Malaysia, Indonesia and the Philippines. It is a tropical genus with 196 species of mainly rainforest trees, out of which 148 species are currently listed in the IUCN Red List; majority of them are listed as critically endangered (Savithamma, 2011). Many species are economically important timber trees. *Shorea* species are found on the borderline between the moist fertile evergreen forests and the less moist and dry deciduous forests in India. The *Shorea* species found in India are *S. assamica*, *S. robusta*, *S. roxburghii* and *S. tumbuggaia*. (Madhavachetty, *et al*, 2008; Ankanna, *et al*, 2011)

Holostemma ada Kodienschult (Asclepiadaceae) is an important medicinal plant and widely distributed in tropical forest in India (Kolamall,

1979). The plant is used as antidiabetic, rejuvenative, aphrodisiac, expectorant, intestinal disorders, galactagogue, stimulant, Orchitis, pain, stomach ache, gonorrhoea and in ophthalmic disorders (Warrier, *et al*, 1995; Chhpr, *et al*, 1956). There is a huge demand for this plant; more than 150 tonnes required every year in south Indian pharmacies. The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites. Hence the present work is focused on *in-vitro* anthelmintic activity assessment on the leaf extracts of *Shorea tumbuggaia Roxb.* and *Holostemma ada Kodienschult*.

MATERIALS AND METHODS

Plant material

Leaves of *Shorea tumbuggaia Roxb.* and *Holostemma ada Kodienschult* from Tirumala hills, Chittoor District, Andhra Pradesh, India; and the plant material was identified and authenticated by Dr. Madavashetty, Professor, Department of Botany, S.V. University, Tirupathi (A.P.), and India. The collected materials were washed thoroughly and kept shade dried individually.

Animals

Indian adult earth worm (*Pheretima posthuma*) was collected and identified from Department of Zoology, S K University, Ananthapuramu and Department of Soil and Fertilization, Ananthapuramu, A.P. India.

Drugs and chemicals

The following drugs and chemicals were used. Drugs: Albendazole tablet, 400mg (BANDY, Mankind Pharma Ltd., New Delhi), Chemicals: Methanol A.R. (Finar chemicals, Pune), ethyl acetate (SD Fine Ltd, Mumbai), Sodium chloride (Finar chemicals, Pune), Distilled water.

Procedure for Extraction of *Shorea tumbuggaia Roxb (STR)* and *Holostemma ada Kodien Schult (HAKS)* of plant material

The leaves of both *Shorea tumbuggaia Roxb (STR)* and *Holostemma ada Kodien Schult (HAKS)* after shade dried were pulverized by a power-driven grinder and the powder were passed through sieve (40-mesh), to get a fine powder of STR and HAKS individually. 2kg powder material of each plant material, were subjected for sequential extraction individually with increase in the polarity of the solvents n-hexane, ethyl acetate, methanol, and hydro-alcohol (1:1) at 60-70°C by Soxhlet extractor. All the extracted solvents were concentrated under reduced pressure using Heidolph rotary evaporators. These extracts were subjected for phytochemical screening by employing standard phytochemical tests (Treare, *et al*, 1982).

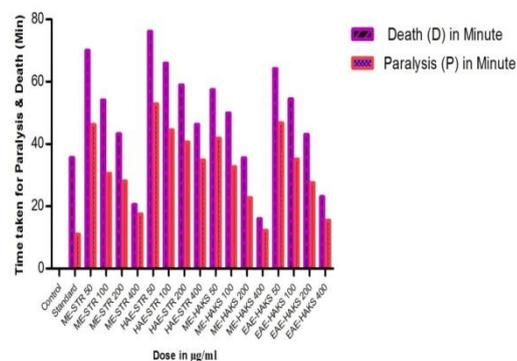
Screening for In-vitro Anthelmintic Assay

The Anthelmintic assay was carried out as per (Ajaiyeoba, *et al* 2001) the method. The assay was performed in vitro using adult Indian earthworm and *Pheretima posthuma* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation Anthelmintic activity (Vidarthi, 1967). Earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. In-vitro bio assay was carried out using, six groups of six earthworms worms i.e. *Pheretima Posthuma* approximately equal size (6-8 cm) were released in to 25 ml of solutions of Albendazole, and various concentration of extracts (ME-STR, HAE-STR, ME-HAKS and EAE-HAKS) in the range of 50, 100, 200, and 400 µg/ml were prepared using with normal saline on to a Petri dish. Albendazole was used as reference standard and normal saline as control. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted

when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously colour of the body nor when dipped in warm water (50°C).

RESULTS

In-vitro Anthelmintic activity of leaf extracts of *Shorea tumbuggaia Roxb. Holostemma ada Kodien Schult.*



Control (Normal Saline) Standard (Albendazole 10µg/ml), ME-methanolic extract, HAE- hydro alcoholic extract, EAE- ethyl acetate extract of *Shorea tumbuggaia Roxb (STR)* and *Holostemma ada Kodien Schult. (HAKS)* concentration ranging from 50, 100, 200 & 400 µg/ml. *** P < (0.0001) compared with the standard; Analysed by using student't' - test one-way ANOVA.

The results of anthelmintic studies reports for the leaf extracts of STR and HAKS are given below.

Fig.1: In-vitro Anthelmintic activity of leaf extracts of *Shorea tumbuggaia Roxb. & Holostemma ada Kodien Schult.*

Table 1: In-vitro Anthelmintic activity of leaf extracts of *Shorea tumbuggaia Roxb. & Holostemma ada Kodien Schult.*

S. No	Treatment	Dose (µg/ml)	Time Taken For Paralysis (Min) MEAN±SD	Time Taken For Death (Min) MEAN±SD
1	Control (Normal saline)	---	---	---
2	Standard (Albendazole. Tab)	10	10.983± 0.765	13.73 ± 1.15
3	Methanolic Extract <i>Shorea tumbuggaia Roxb</i>	50	46.233± 2.444	69.96 ± 5.20
		100	30.517± 13.441	54.02 ± 2.12
		200	28.100± 0.837	43.22 ± 2.33
		400	17.467± 1.525	20.53 ± 1.14
4	Hydroalcoholic Extract <i>Shorea tumbuggaia Roxb</i>	50	52.783± 2.469	76.07 ± 2.27
		100	44.450± 2.067	65.90 ± 2.92
		200	40.567± 0.763	58.92 ± 2.79
		400	34.733± 1.519	46.20 ± 1.91
5	Ethyl Acetate Extract <i>Holostemma ada Kodien Schult.</i>	50	46.783± 2.154	64.15 ± 1.64
		100	35.083± 1.419	54.42 ± 1.51
		200	27.550± 2.147	43.05 ± 0.93
		400	15.450± 1.981	23.05 ± 1.66
6	Methanolic Extract <i>Holostemma ada Kodien Schult.</i>	50	41.850± 1.545	57.36 ± 3.21
		100	2.600 ± 1.893	49.90 ± 1.34
		200	22.683 ± 1.883	35.53 ± 3.55
		400	12.267 ± 1.707	15.93 ± 3.18

Control (Distilled water) Standard (Albendazole 10mg/ml), ME-methanolic extract, HAE- hydro alcoholic extract, EAE- ethyl acetate extract of *Shorea tumbuggaia Roxb.(STR)* and *Holostemma ada Kodien Schult.(HAKS)* concentration ranging from 50,100,200 & 400 µg/ml. *** P < (0.0001) compared with the standard; Analysed by using student't' - test one-way ANOVA.

DISCUSSION

Preliminary phytochemical screening shows the presence of presence of flavonoids, anthocyanins, tannins, saponines and phenols in *Shorea tumbuggaia Roxb. (STR)*; and presence of flavonoids, tannins, saponines, anthocyanins, alkaloids, amino acids, reducing sugars and steroids in *Holostemma ada Kodien Schult.(HAKS)*. From our previous experiments made on all the

extracts of both was subjected for antioxidant activity, total phenol and flavonoids content (data not included) [Rubesh Kumar, *et al*, 2014]; the studies proves that the presence of the strong antioxidant activity, phenolic and flavonoidal composition was enriched in the (ME-STR, HAE-STR, ME-HAKS and EAE-HAKS) extracts. The selected extracts were subjected for the in-vitro assessment of anthelmintic activity. In the past tannins have been scientifically confirmed to possess the anthelmintic activities (Niezen, *et al*, 1995); chemically

these tannins are polyphenolic compound and reports were recorded for some synthetic phenolic anthelmintic interferes with energy of helminth parasites by uncoupling oxidative phosphorylation (Bate-Smith, 1962). In- vitro anthelmintic results shown in (Table 1). The predominant effect of Albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis (Martin, 1997; Chatterjee, 1967; Thorn, et al, 1977; Vigar, 1984). From the result it is evident that various concentration (50,100, 200, and 400 µg/ml) of the leaf extract of *Shorea tumbergaia* Roxb. and *Holostemma ada kodien* Schult. Showed paralytic and death time as the dose -dependence. Paralytic effect (P) of was witnessed for both methanolic extract ME-STR, EAE-HAKS and ME- HAKS (17.467± 1.525, 15.450± 1.981 and 12.267 ± 1.707) extract showed potent activity and HAE-STR (34.733 ± 1.519) when compared with reference standard respectively, but the death time (D), were faster for the methanolic extract of both the plants 15.93 ± 3.18 (ME-HAKS) and 20.53 ± 1.14 (ME-STR) ; 23.05 ± 1.66 for the (EAE-HAKS) extract respectively. All results was statistically analysed using Analysed by using student 't' - test one-way ANOVA; the P < (0.0001) were significant when compared with standard group (Figure 1).

CONCLUSION

It can be concluded that the present report confirms that the methanolic extract of *Shorea tumbergaia* Roxb. and methanolic and ethyl acetate extract of *Holostemma ada kodien* Schult. shows a potent anthelmintic activity. The results of paralysis time and death time of methanolic extract of both the plant were showing greater activity at concentration-dependent when compared with standard albendazole. Phytochemical screening reveals that the presence of phenolic and its sub classified constitution were reported for its anthelmintic activity by the innumerable investigators in natural products. From our previous data both the plants exhibits the potent antioxidant, and presence of total phenol and flavonoidal compound. Thus, the isolation, purification and characterization of phenols and its related compound may be new chemical entities which may be less toxic can be used for the treatment of helminthiasis.

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