

**ANTHELMINTIC EFFICACY OF SOME INDIGENOUS PLANTS
USED IN THE TRADITIONAL REMEDIES OF NAGA TRIBES**

ABSTRACT

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Abstract

The present work incorporates a study on ascertaining the anthelmintic activity of seven medicinal plants that are commonly used in the folklore medicine system of Naga tribes in Nagaland to cure helminthic parasitic infections. The objectives of the study were:

1. to test the anthelmintic efficacy of some folklore medicinal plants used in the traditional medicine system of Naga tribes.
2. to compare the anthelmintic efficacy of these plants with broad-spectrum anthelmintic drugs.
3. to investigate the acute toxicity of plants in experimental animals.
4. to investigate the effects of these plant extracts on surface fine topography of parasites.

To evaluate the anthelmintic activity of folklore medicinal plants, seven plant species, namely - *Centella asiatica* L. (Apiaceae), *Clerodendrum colebrookianum* Walp. (Verbenaceae), *Curcuma longa* L. (Zingiberaceae), *Gynura angulosa* DC. (Asteraceae), *Houttuynia cordata* Thunb. (Piperaceae), *Lasia spinosa* L. (Araceae) and *Psidium guajava* L. (Myrtaceae) were included based upon the information collected about their use as deworming agents from the traditional practitioner and local people in the Nagaland state. The various usable plant parts were extracted in methanol and the crude extracts were

tested at different concentrations *in vitro* and *in vivo* against several helminth parasites. For *in vitro* study, *Raillietina echinobothrida*, *Hymenolepis diminuta*, *Gastrothylax crumenifer*, *Ascaridia galli* and *Trichinella spiralis* served as the test parasites. While *Hymenolepis diminuta* - rat and *Trichinella spiralis* - mice animal models were involved to evaluate the *in vivo* anthelmintic efficacy of plant extracts.

In the *in vitro* studies the test parasites were exposed to 5, 10, 20 and 40 mg/ml concentrations of plant extracts and mortality of worms served as the anthelmintic criterion. In each case the parasites were also exposed to corresponding concentrations of a standard anthelmintic drug to compare the efficacy of plant extract. Out of the seven plant extract tested, *P. guajava*, *H. cordata*, *L. spinosa* (stalk and leaf), *G. angulosa* and *C. colebrookianum* revealed significant anthelmintic efficacy. However, a moderate level of anthelmintic efficacy was observed for *L. spinosa* (stem), *C. asiatica* and *C. longa*. With respect to various helminthic groups, the study revealed that the leaf extract of *P. guajava*, *H. cordata* and stalk of *L. spinosa* possess profound efficacy against the cestode parasite, *R. echinobothrida*. The leaf extract of *P. guajava*, *L. spinosa* and *G. angulosa* manifested appreciable anticestodal efficacy against *H. diminuta*. Of different plant extracts tested against *G. crumenifer*, leaf extracts of *L. spinosa*, *C. colebrookianum* and *H. cordata* showed good flukicidal efficacy. In case of roundworm *A. galli*, only the leaf extract of *L. spinosa* was found to possess promising anthelmintic activity. Lastly, against the adult *T. spiralis* worms leaf extracts of *G. angulosa*, *L.*

spinosa, *C. colebrookianum*, *H. cordata* and *P. guajava* revealed significant activity. The individual plant extracts showing significant efficacy were further tested in combination with other extracts to investigate whether they could have any synergistic effects on mortality of parasites. No substantial increase in the anthelmintic efficacy of extracts was observed in such investigations.

The present study revealed that *P. guajava* leaf extract possess significant level of efficacy against *R. echinobothrida*, *H. diminuta* and *T. spiralis*. In case of its efficacy against *R. echinobothrida*, both its 20 and 40 mg/ml concentrations revealed the mortality of parasites in 1.00 h. Against *H. diminuta*, the extract showed mortality of worms in 2.34 h at 40 mg/ml concentration. Mortality of *T. spiralis* in its 40 mg/ml concentration was observed to be in as early as in 0.92 h. The *H. cordata* extract showed significant *in vitro* anthelmintic efficacy against *R. echinobothrida*, *G. crumenifer* and *T. spiralis*. *R. echinobothrida* treated with the 40 mg/ml concentration of *H. cordata* extract showed mortality of worms within 2.00 h. The efficacy of extract was recorded to be slightly lower against *G. crumenifer*, wherein it caused mortality of worms in 3.00 h. Against *T. spiralis*, the 40 mg/ml concentration of extract showed mortality of worms in as early as in 0.89 h.

In the present study the *L. spinosa* leaf extract showed profound anthelmintic efficacy against *H. diminuta*, *G. crumenifer*, *A. galli* and *T. spiralis*. The *H. diminuta* worms showed mortality within 2.50 h at its 40 mg/ml

concentration. The amphistome, *G. crumenifer* exposed to 40 mg/ml concentration of extract revealed the mortality of worms in 2.09 h which was almost comparable to Praziquantel (PZQ), the reference drug. The stalk extract of *L. spinosa* was also evaluated for anthelmintic efficacy in the present study and showed good efficacy only against *R. echinobothrida*. Whereas the stem extract of *L. spinosa* was not found to be as effective as leaf and stalk extract.

The present investigation revealed that *G. angulosa* possesses prominent anthelmintic activity only against *H. diminuta* and *T. spiralis*. The plant extract at 40 mg/ml concentration showed mortality of *H. diminuta* worms in 2.92 h compared to PZQ which showed mortality of parasites in 0.60 h at the same concentration. Similarly, for *T. spiralis* also the plant extract showed almost comparable efficacy with that of reference drug, Mebendazole (MBZ).

C. colebrookianum extract showed significant level of efficacy against *G. crumenifer* and *T. spiralis*. At 40 mg/ml concentration the efficacy of *C. colebrookianum* extract and reference drug was almost similar. The mortality time of parasites at this concentration was recorded to be 2.50 h and 2.10 h, respectively. The *C. colebrookianum* extract, however did not show notable efficacy against *R. echinobothrida*, *H. diminuta* and *A. galli*.

In the present study *C. asiatica* leaf extract showed moderate level of efficacy against *R. echinobothrida*, *G. crumenifer* and *A. galli* and rather

insignificant efficacy against *H. diminuta* and *T. spiralis*. Unlike other tested plant extracts, *C. longa* extract did not show anthelmintic efficacy worth pursuing further.

The parasites' tegument/cuticle has been implicated among one of several target sites by which natural anthelmintic products or synthetic drugs act. In the present study effects of selected plant extracts were studied on parasite body surface with the help of scanning electron microscopy (SEM) so as to provide some clues regarding their plausible mode of action. The study revealed that barring *H. cordata* other extracts, namely *L. spinosa*, *G. angulosa*, *P. guajava* and *C. colebrookianum* exhibited such morphological changes and damage to the parasite's body surface. The tegument of *H. diminuta* and *G. crumenifer* showed destruction in the form of erosion on all over the general topography of the body. In case of *Hymenolepis* the scolex also showed apparent damage. Similarly, for *A. galli* the SEM of extract treated worms revealed wrinkles and cracks on lips and body cuticle.

To further substantiate the efficacy of plant extracts, the *in vitro* studies were supplemented with *in vivo* studies wherein the plant extracts were also tested for their anthelmintic efficacy in *H. diminuta* - rat and *T. spiralis* - mouse experimental models. In *Hymenolepis* - rat model the extracts were administered at three different stages of parasites; the larval, immature and adults. Efficacy was adjudged by counting the eggs per gram of faeces (EPG), worm reduction

and host clearance rate. In all experiments Praziquantel, a broad spectrum anthelmintic drug was tested at 5 and 10 mg/kg, p.o. doses as a reference drug. The results indicated that there were significant changes in all these parameters in the treated groups of animals as compared to untreated control. With respect to efficacy of extracts against larval stages, more prominent effects were recorded for *P. guajava*, *L. spinosa* (leaf), *H. cordata*, *C. longa* and *G. angulosa* extracts. The treatment of rats with 1600 mg/kg doses of the above plant extracts on days 2-6 p.i. resulted in elimination of 66.50, 66.66, 62.50, 62.50 and 58.25% of adult worms, respectively. Administration of extract on days 21-25 p.i. to investigate the efficacy against the adult stage showed percentage worm reduction between 87.50 to 91.50% for the tested plant extracts. The extract treated group of animals also showed substantial decrease in EPG values. The acute toxicity study in the experimental rats showed that barring mortality of few animals as noticed in the *C. longa* and *L. spinosa* stem extract-treated groups no other plant extracts cause any mortality or any changes in behaviour of animals with regard to food and water intake.

The efficacy of extracts in *T. spiralis* - mouse model was investigated against the adult, migrating and encysted stages; percentage reduction in adult worms at necropsy or larvae encysted in tissue constituted the study parameters. In general, barring *C. asiatica*, all other plant extracts tested in this study showed moderate to high efficacy against the adult *Trichinella* worms and more or less similar was the case against their efficacy against the migrating

larvae. In contrast, barring *G. angulosa* the rest of the plant extracts showed either medium or very low level of efficacy against the encysted larvae. The leaf extract of *G. angulosa* showed up to 86.22% of adult worm reduction and 72.36% encysted larvae reduction. The efficacy when compared to 25 mg/kg dose of Mebendazole, the reference drug was noted to be 94.70 and 90.52%, respectively against these stages. The acute toxicity studies of extracts in mice showed maximum mortality of animals for *C. longa* extract, followed by *C. colebrookianum* and *H. cordata* extracts. However, the rest of the plant extracts neither caused any mortality nor any visible signs of toxicity in experimental animals.

This study thus validates the presence of appreciable anthelmintic property in many of the folk medicinal plants used by Naga tribes which may have therapeutic benefits in humans encountering helminthic infections. Further investigation on isolated chemical constituents of these plants should be pursued against different helminth parasite species.

Two photographic plates of seven plants, seven photographic plates of twenty five scanning electron micrograph pictures, twenty graphic figures, two life cycle diagrammatic figures and thirty nine tables support the study observations carried out in the present work. Altogether (176) citations are given in the references.