An Endangered Medicinal Plant, *Ilex khasiana* Exhibits Potent Antiparasitic Activity Against Intestinal Tapeworm

Charles Lalnunfela1,2, P. B. Lalthanpuii1, T. C. Lalhriatpuii1, K. Lalchhandama2,**

**ABSTRACT**

Background: A species of holly, *Ilex khasiana* Purk. (family Aquifoliaceae) is one of a few critically endangered medicinal plants in India. It is endemic to Khasi Hills, Meghalaya, and Aizawl, Mizoram. In the traditional medicines of Khasi and Mizo people, the leaves are used as a panacea for all sorts of infections. Nothing is known about its true pharmacological potentials.

Objective: We aimed to assess the extract of *I. khasiana* leaves for its purported antiparasitic property. To test the antiparasitic activity, we collected intestinal tapeworms, *Raillietina tetragona*. Materials and Methods: *I. khasiana* leaves were dried and extracted in methanol. The plant extract was used to treat the tapeworms at different concentrations. The efficacy was recorded as survival values and were analysed by Student's *t*-test. The plant extract-treated tapeworms were fixed, dehydrated and observed under scanning electron microscopy for studying the structural details. Results: The methanol extract of *I. khasiana* leaf showed dose-dependent antiparasitic effect as that of albendazole. The treated tapeworms developed massive structural damages. The scolex was deformed, with its tegument extensively damaged. The suckers had their spines clumped. The body segments severely contracted and some portions were eroded. Hair-like microtriches were completely lost. Conclusion: Our findings indicate that *I. khasiana* has antiparasitic activity and requires further investigations, and that there is a need to foster its conservation and propagation.

Key words: Medicinal plant, Parasite, Scanning electron microscopy, Tegum, Tapeworm.

**INTRODUCTION**

With almost 600 species described so far in the family Aquifoliaceae, *Ilex* is the only surviving genus. Commonly known as holly, species of *Ilex* are familiar in traditional Chinese medicine, especially *I. kudingcha* and *I. latifolia*, the leaves of which are parts of dietary foods. They are used as therapeutic agents for their analgesic, antiarrrheoal, antipyretic, astringent, haematostatic, and hepatoprotective properties.1 South American natives use *I. paraguariensis* leaves as health-benefit drink and are now commercialised as mate tea. The drink is acclaimed for its hepatoprotection, digestive, anti-rheumatism, and anti-obesity properties, as well as its ability to prevent arthritis, inflammation, hypertension and hypercholesterolemia.2 Bioactive compounds from the plant are experimentally validated to have antiinflammatory,1 anticancer,1 anti-obesity,3 and cholesterol-reducing activities in animal models.6

*I. pubescens* is also well known in traditional Chinese medicine for the treatment of coronary heart diseases, and as antiinflammatory and analgesic agents.1 It is also known to be an effective remedy for hypertension, hyperlipemia, and hepatitis.7 Compounds from its root are demonstrated as having antiocoagulant,8 antiinflammatory,9 and neuro-protective activities.10 *I. cornuta* is used in the treatment for dizziness and hypertension.11 Saponins from its roots are validated as possessing antiviral activity against herpes simplex virus type 1,12 and myocardial (heart tissue) damage inhibition.13 *I. ficoides* and *I. centrochinensis* were shown to have potent antiinflammatory and antioxidant activities.14

Out of 410 accepted species recorded in the genus *Ix*, *I. khasiana* Purk. is outstanding in that it is critically endangered according to the IUCN Red List of Threatened Species,15 and that its cohort of medicinal properties attracts the least interest so far. It is reported as endemic to the Khasi Hills of Meghalaya, northeast India. About 3,000 individual trees are estimated to exist in these areas.16 But an isolated distribution is also now recorded at Luangmual, Aizawl, Mizoram. Among the Khasi people, a decoction of the bark and the root is used in the treatment of tuberculosis and severe cold.17 The leaves are known for various medicinal properties including antiparasitic property in Mizo traditional medicine. In fact, the Mizo traditional practitioners use them as a panacea in all sorts of non-specific illnesses.18 It is therefore important to understand the true pharmacological potentials of this plant as an antiparasitic agent.

**MATERIALS AND METHODS**

Plant specimen and preparation of plant extract

*Ilex khasiana* is available only at one area in Mizoram, India, as naturally propagated tree at Luangmual, Aizawl (location 23°44.85′N and 92°41.95′E). The plant specimen was authenticated at the Botanical Survey of India, Eastern Circle, Shillong, Meghalaya.
The antiparasitic activity of the methanol extract of I. khasiana leaves and albendazole on the tapeworm, Raillietina tetragona, is given in Table 1 and Figure 1. Tapeworms in the control (negative treatment) media could survive up to 74.03 hr. Survival values were normalised against that of the control. At the concentrations of 1.25, 2.5, 5, 10 and 20 mg/ml, the plant extract took 56.42 ± 1.73, 46.72 ± 2.01, 37.08 ± 1.98, 29.25 ± 1.76, and 20.40 ± 2.55 h to kill all the parasites. Whereas the drug albendazole took 23.76 ± 1.93, 20.24 ± 0.58, 16.30 ± 0.66, 12.15 ± 0.61, and 4.39 ± 0.88 hr respectively for killing the tapeworms.

Scanning electron microscopy revealed details of structural changes on the tapeworms treated with 20 mg/ml of I. khasiana leaf extract. Figure 2 is the anterior part of the tapeworm consisting of the bulb-like scolex and the adjoining neck. Large folds were visible at the base of the scolex and on the neck showing tegumental degeneration at the anterior end of the body. A single sucker shown in Figure 3 indicates tegumental shrinkage and disintegration of the spines around the rim. A magnified view of the same sucker (Figure 4) revealed disordered and clumping of the spines and debris-like tegumental erosion.

The main body (strobila) of tapeworms is a series of body segments (proglottids). All the body segments exhibited irregular shrinkage and disintegration of the spines around the rim. A magnified view of the same sucker (Figure 4) revealed disordered and clumping of the spines and debris-like tegumental erosion.

The antiparasitic activity against the methanol extract of I. khasiana leaves and albendazole on the tapeworm, Raillietina tetragona, is given in Table 1 and Figure 1. Tapeworms in the control (negative treatment) media could survive up to 74.03 hr. Survival values were normalised against that of the control. At the concentrations of 1.25, 2.5, 5, 10 and 20 mg/ml, the plant extract took 56.42 ± 1.73, 46.72 ± 2.01, 37.08 ± 1.98, 29.25 ± 1.76, and 20.40 ± 2.55 h to kill all the parasites. Whereas the drug albendazole took 23.76 ± 1.93, 20.24 ± 0.58, 16.30 ± 0.66, 12.15 ± 0.61, and 4.39 ± 0.88 hr respectively for killing the tapeworms.

### Table 1: Antiparasitic activity of I. khasiana leaf extract and albendazole on R. tetragona.

<table>
<thead>
<tr>
<th>Media</th>
<th>Dose (mg/ml)</th>
<th>Normalised survival time in hr (± SD)</th>
<th>t value</th>
<th>t critical value</th>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>100.00 ± 2.56</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Albendazole</td>
<td>1.25</td>
<td>023.76 ± 1.93</td>
<td>58.32</td>
<td>2.26*</td>
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<tr>
<td></td>
<td>2.5</td>
<td>020.24 ± 0.58</td>
<td>74.53</td>
<td>2.45*</td>
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<tr>
<td></td>
<td>5</td>
<td>016.30 ± 0.66</td>
<td>77.66</td>
<td>2.45*</td>
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<tr>
<td></td>
<td>10</td>
<td>012.15 ± 0.61</td>
<td>81.85</td>
<td>2.45*</td>
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<tr>
<td></td>
<td>20</td>
<td>004.39 ± 0.88</td>
<td>86.57</td>
<td>2.45*</td>
</tr>
<tr>
<td>I. khasiana leaf extract</td>
<td>1.25</td>
<td>056.42 ± 1.73</td>
<td>34.57</td>
<td>2.26*</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>046.72 ± 2.01</td>
<td>40.14</td>
<td>2.26*</td>
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<tr>
<td></td>
<td>5</td>
<td>037.08 ± 1.98</td>
<td>47.62</td>
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<td>10</td>
<td>029.25 ± 1.76</td>
<td>55.78</td>
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<tr>
<td></td>
<td>20</td>
<td>020.40 ± 2.55</td>
<td>53.96</td>
<td>2.23*</td>
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</table>

*Significantly different at p < 0.05 in comparison with control (negative treatment) group; n = 6.
DISCUSSION

Tapeworms are a group of helminth parasites which are largely ignored and often described as “low priority” infectious agents due to their relative quiescence in most infections. However, they pose serious threats to clinical conditions such as neurocysticersosis in humans, livestock production, and wildlife. They are distinct helminths in having rather modest anatomical organisation; lack of nervous and digestive systems being the unique features. Their external body surface called tegument is the principal body organ as it serves not only as a protective barrier, but also as nutrient-absorptive and sensory site. These functions are carried out through microscopic hairs called microtriches, which are distributed throughout the tegument. Thus, the hallmark effects of antiparasitic drugs are structural damages on the tegument, microtriches and the underlying subtegument tissue.

The detail structural features of the tegument and its physical and physiological roles in poultry tapeworms are already amply described. In our experiment, the methanol extract of *I. khasiana* leaves caused considerable tegumental damages including shrinkage, distortion of the spines and rostellum, and erosion of the microtriches. The antiparasitic activity was further substantiated by concentration-dependent lethal activity on *R. tetragona* similar to that of albendazole. Benzimidazoles are the drugs of choice in all types of helminth infection because of their broad-spectrum activity. The most commonly used...
benzimidazoles, albendazole and flubendazole cause degeneration of the rostellum, destruction of the microtriches, and eruption of aberrant vesicles on the host body, Echinococcus granulosus. A combination therapy of albendazole and praziquantel resulted in disintegration of the suckers, detachment of the spines, and severe damage of the tegument, associated with removal of the microtriches in E. granulosus and Mesocestodes corti. A single treatment of R. echinobothrida with albendazole produced severe tegumentary contraction and collapse. In addition, the suckers were destroyed while the rostellum remained intact.

Nitazoxanide also induced pore formation on the socalled and distortion of the neck and the strabular chains of the immature (cysticerci) of Taenia solium. E. granulosus and E. multilocularis treated with lonidamine and 6-aminonicotinamide showed tegumental darkening, swelling and shortening of the body segments, surface erosion, and open a new vista for the development of novel antiparasitic drug. R. tetragona. The clumping of spines leaf extract indiscriminately exhibit variation in its effect on the intensity and extend of tegumental damage of the tegument, associated with removal of the microtriches in E. granulosus and Mesocestodes corti. A single treatment of R. echinobothrida with albendazole produced severe tegumentary contraction and collapse. In addition, the suckers were destroyed while the rostellum remained intact.

CONCLUSION

We found that I. khasiana leaf extract effectively killed the tapeworm R. tetragona. It caused structural damages on the tegument of the tapeworm, indicating the characteristic antiparasitic effects. Under scanning electron microscopy, distortion of the microtriches, clumping of the spines, erosion of the microtriches and extensive contraction of the tegument were evident. The need for conservation and propagation of this valuable medicinal plant is compelling. Further studies on the chemical components of the medicinal plant, isolation of the active targets. Parasitology Research. 2014;113(7):2425-2433.

CONFLICTS OF INTEREST

None declared.

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REFERENCES

A species of holly, *Ilex khasiana*, is a critically endangered medicinal plant that is known to have a variety of uses in traditional medicine. Among its medicinal properties is antiparasitic activity.

The methanol extract of the leaves was tested in vitro against an intestinal tapeworm, *Raillietina tetragona*. The plant extract caused dose-dependent antiparasitic activity against the tapeworm as that of albendazole.

Scanning electron microscopy was used to examine structural changes on the tapeworm. The body surface (tegument) indicated damages which are characteristics of antiparasitic drugs such as shrinkage, erosion, disintegration of the suckers, and removal of microtriches.

The damaging effects imply that the plant possesses bioactive compounds that can be new lead molecules in drug development. Thus, the findings warrant further investigations on the chemical nature and precise mode of action.

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