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Effect of root-tuber extract of *Flemingia vestita*, a leguminous plant, on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski*: a scanning electron microscopy study

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Abstract The tegumental surface of *Artyfechinostomum sufrartyfex* as viewed under the scanning electron microscope revealed the presence of double rows of spines in the collar. The dorsal surface (6–8 rows) and the ventral surface are provided with posteriorly directed spines. The normal body surface of *Fasciolopsis buski* shows posteriorly directed scales throughout the ventral surface; the dorsal surface is free of any scales but has domed, coarsely distributed papillae. When treated in vitro with ethanol root-tuber extract of *Flemingia vestita*, an indigenous medicinal plant in Meghalaya, India, at a concentration of 5, 10, and 20 mg/ml phosphate-buffered saline (PBS), *A. sufrartyfex* became paralyzed within 1.1–1.4, 0.8–1.0, and 0.3–0.5 h, respectively. Following similar treatment, *F. buski* took 3.0–3.6, 1.5–2.0, and 0.6–0.8 h, respectively, to reach a paralytic state. Oxyclozanide B.P. was used as the reference drug and paralyzed the worm, taking slightly less time than the crude extract for both species of flukes. Stereoscanning observations on the tegumental surface of treated (20 mg extract/ml PBS) *A. sufrartyfex* revealed sloughing off of most of the spines or their deformation as well as wrinkles and rupture of the general tegument. Severe tegumental alterations and deformities were also displayed by *F. buski* exposed to 20 mg extract/ml PBS.

Introduction

Plant products provide and are gaining importance as an alternative to current medical practices involving chemotherapy (Didier et al. 1988; Robinson et al. 1990). For many reasons, the latter may not be accessible to the masses in developing and underdeveloped countries. In Northeast India, Meghalaya in particular, many indigenous plants are used for their medicinal value (Rao 1981). *Flemingia vestita* Benth and Hooker (family Leguminoseae), locally known as Soh-phlang, is widely used by the native tribal population of Meghalaya, who consume the pulpy tuberous roots for their anthelmintic activity against intestinal worm infections. In a preliminary study, the whole root-tuber crude extract has been reported to be effective against *Ascaris suum* in vitro (Yadav et al. 1992). The active principles of the root-tuber peels have been isolated by Rao and Reddy (1991); these are isoflavones and have been identified as genistein (0.25), fomononetin (0.035%), pseudobaptigenin (0.015%), and daidzein (0.01%).

The present study aimed at testing the efficacy of root-tuber extract of *F. vestita* on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski*, both of which are intestinal digenetic flukes occurring in pigs but also having a zoonotic potential. It pertains to scanning electron microscopic observations on the surface fine topography and its alterations induced by the plant crude extract on in vitro exposure. Microtopographical details of *F. buski* have previously been studied (Roy and Tandon 1993), whereas those of *A. sufrartyfex* are reported herein for the first time.

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Materials and methods

Preparation of crude extract

The edible root tubers of *Flemingia vestita* Benth and Hooker were collected from neighboring villages of Shillong, India, during November 1993, and their peels were dried at 50° C in an oven. Dried peels (about 100 g) were ground and put in a reflux flask having 1 l capacity with 500 ml rectified spirit. After reflux for 8 h at 60° C, the solution was filtered and dried overnight at 60° C; 26 g crude extract was obtained from 100 g dried peels.

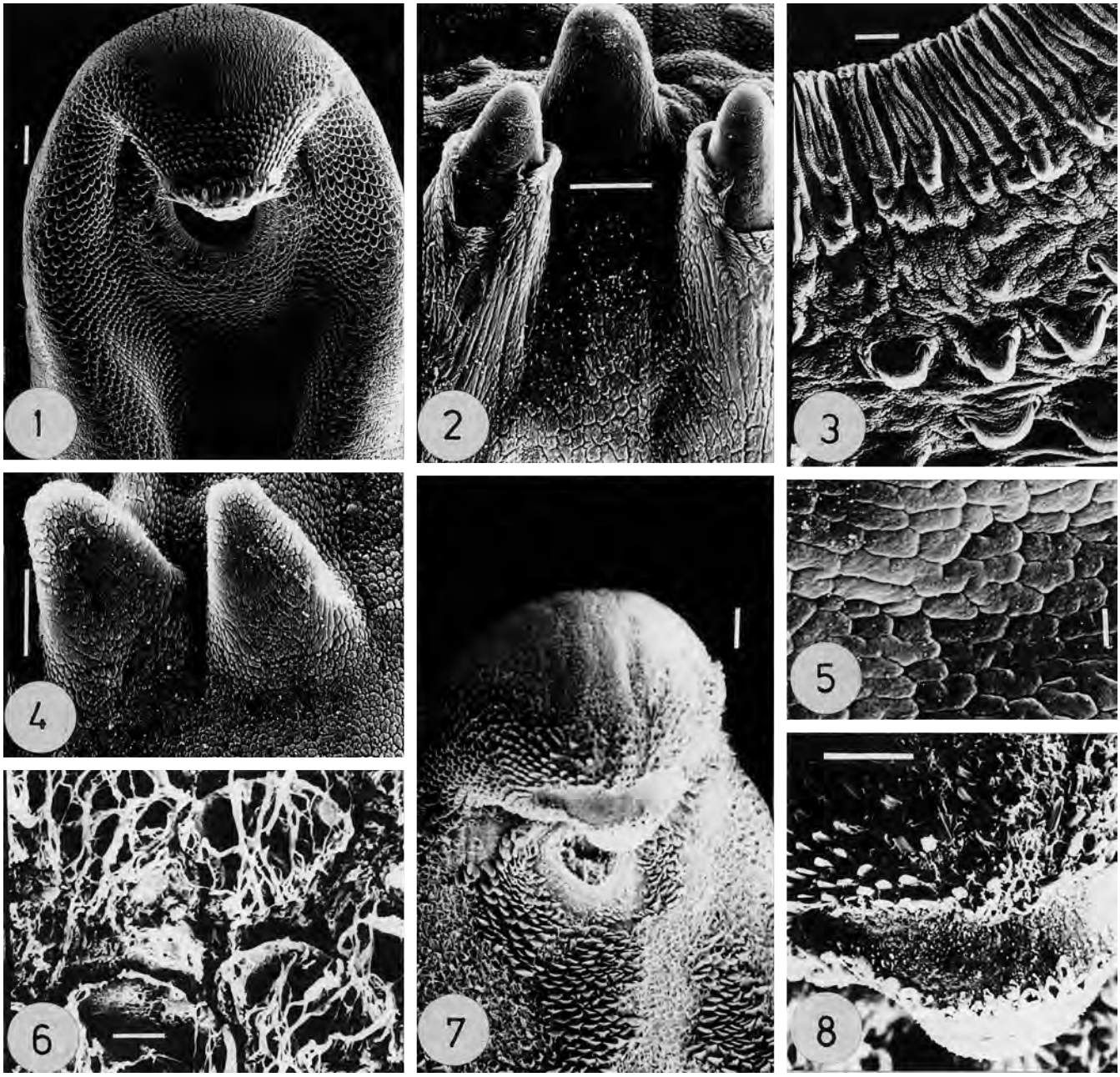
Experimental flukes and bioassay

Adult *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski* were collected from the intestine of a locally reared pig at an abattoir in Shillong. After a washing in phosphate-buffered saline (PBS), the flukes were incubated at 37±1° C with varying concentrations of

the extract, viz., 5, 10, and 20 mg/ml PBS (three replicates for each concentration) in 1% dimethylsulfoxide (DMSO). Control incubation consisted of flukes in PBS with 1% DMSO only. Oxyclozanide B.P., a wide-spectrum flukicide,was used as the reference drug at concentrations similar to those used for the crude extract.

Scanning electron microscopy

Flukes were fixed in 10% neutral buffered formalin at 4° C for 4 h, dehydrated in acetone, and air-dried in tetramethylsilane (Roy and Tandon 1991). The gold-coated specimens were viewed under a Jeol JSM-35CF electron microscope at an electron-accelerating voltage of 10–15 kV.



Figs. 1–8 *Artyfechinostomum sufrartyfex* – scanning electron micrographs of normal (**Figs. 1–5**) and treated (**Figs. 6–8**) flukes

Fig. 1 Anterior half of a whole worm, ventral view. Bar=100 μm

Fig. 2 Collar spines in a closer view, revealing the socketed and nonsocketed types. Bar=10 μm

Fig. 3 Portion of the ventral sucker rim and adjoining ventral surface. Bar=10 μm

Fig. 4 Dorsal spines in an enlarged view. Bar=10 μm

Fig. 5 Dorsal surface (general tegument). Bar=1 μm

Fig. 6 Portion of the ventral surface, showing pits and disrupted tegument. Bar=10 μm

Fig. 7 Anterior end of a worm (ventral view). Bar=100 μm

Fig. 8 Enlarged view of the collar region, showing deep pits in areas of spines and scales. Bar=100 μm