SUMMARY

Global concern over rapid evolution of drug resistance in helminth parasites and general endorsement of organic farming pose serious constraint on prolonged application of pharmaceutical anthelmintics. An alternative includes experimental evaluation of medicinal plants used in traditional practices as remedy to worm infections. Among a number of ethnomedicinal plants used by the Mizo tribes of north-east India, parts of two leguminous plants, *Acacia oxyphylla* Graham ex Bentham, known to the Mizo natives as ‘khangngo,’ and *Millettia pachycarpa* Bentham, ‘Rulei’ to the natives, are employed as therapeutics for intestinal worm infections. To ascertain the traditional usage, extracts from the stem bark of *A. oxyphylla* and the root bark of *M. pachycarpa* were experimentally tested *in vitro* to evaluate whether or not they possess tangible anthelmintic effects on the two major poultry intestinal helminths, the cestode *Raillietina echinobothrida* (phylum Platyhelminthes) and the nematode *Ascaridia galli* (phylum Nematoda). Ethanol, methanol and acetone extracts were prepared from each of the stem bark of *A. oxyphylla* and the root bark of *M. pachycarpa*. The parasites were incubated at 37±1°C with different concentrations (viz 0.5, 1, 2, 5, 10 and 20 mg/ml) of each of the extracts of both the plants prepared in phosphate buffered saline (PBS) supplemented with 1% dimethylsulfoxide (DMSO). Similar doses of albendazole and piperazine hydrate were used as standard anthelmintic drugs for the cestode and the nematode, respectively. Control experiments consisted of worms maintained in only PBS with DMSO at 37±1°C.
R. echinobothrida was highly susceptible to albendazole and all the three extracts of both the plants in terms of motility and mortality effects. Untreated control cestodes survived very well up to 54.78 ± 0.65 hours, while the nematodes could persist for 84.83 ± 0.89 hours. The cestode necessarily underwent a lapse of paralytic stage initially before finally succumbing to actual death upon exposure to the drug and plant extracts. An orderly dose-dependent efficacy was observed for all the test materials \((P < 0.05)\). However, the lowest concentration (0.5 mg/ml) of any of the plant extracts applied did not cause significant lethality. The vitality of A. galli was not significantly affected by the three extracts of M. pachycarpa and the acetone extract of A. oxyphylla. Only the ethanol and methanol extracts of A. oxyphylla at higher concentrations above 2 mg/ml and 5 mg/ml, respectively, could cause simultaneous paralysis and death; while piperazine was highly effective at all concentrations tested.

Scanning electron microscopic observations revealed that extensive deformity occurred on the surface fine topography of R. echinobothrida as a result of treatment with the plant extracts. The three extracts, each at the concentration of 20 mg/ml, of both the plants caused the most intense destruction on the tegumental integrity, however, with slight variations on the nature of the damage. The extracts of A. oxyphylla primarily affected severe shrinkage of the tegument giving rise to series of abnormal folds, lumping of the microtriches, wrinkles on the scolex and distortion of the regular sucker concavity. The ethanol extract effectually excised the spines on the rim of the sucker, while the acetone extract caused the spines to get sharply crooked. Damages due to the extracts of M. pachycarpa were more elaborate generally involving massive eruptions of the tegument, total obliteration of microtrichal filaments, and complete truncation of the suckers and collapse of the spines. The
ethanol and methanol extract treatments resulted in development of cracks and pits on the tegument, associated with surface peeling and erosion. Focal swelling and shrinkage were caused by the acetone extract. Only the 20 mg/ml ethanol and methanol extracts of *M. pachycarpa* affected discernible topographical alterations on *A. galli*. Wrinkles on the lips, shrinkage of the sensory papillae and corrugations of the cuticle were conspicuous at the cephalic region, while prominent focal blebbings were evident throughout the body.

Under light microscopy, structural changes induced by the extracts of *A. oxyphylla* in the internal body organization of *R. echinobothrida* were perceptible as vacuolization in the tegument and subtegument, degeneration of the muscle layer, pygnosis of the egg cells and disintegration of the fine network of the internal parenchyma. Similar destructive effects were noted for the extracts of *M. pachycarpa*; but in addition, removal of large portions of the tegument, generally extending to the subtegument was observed. The magnitude of tissue damage was explicable dose-dependent, and no apparent damage was noticeable for treatments with lower concentrations. Of the extracts of *M. pachycarpa*, only higher concentrations of ethanol and methanol extracts showed noteworthy structural changes in the female nematode. The damaging effects were characterized by degeneration of the cuticular layer, disintegration of the muscle and rupture of ovaries leading to expulsion of eggs. In addition, the ethanol extract effectively damaged the uterine wall, egg membranes and the epithelial linings of the intestine.

Biochemical assays of the levels of vital trace elements such as calcium, magnesium, sodium and potassium, and tegumental enzymes such as acid phosphatase (AcPase) and alkaline phosphatase (AlkPase) of *R. echinobothrida* also revealed that
significant alterations were caused by albendazole and the different extracts of both *A. oxyphylla* and *M. pachycarpa*, at the same concentrations of 20 mg/ml. Significant reduction in the levels of the trace metals and inhibition of the activities of the enzymes were clearly demonstrated, with overall highest efficacy for albendazole, followed by the ethanol extract of *M. pachycarpa*. Comparatively, the different extracts of *M. pachycarpa* were observed to be more potent than those of *A. oxyphylla* in causing these effects. Therefore, it can be concluded that albendazole and the extracts of the two plants exerted their cestocidal activity by drastically decreasing the levels of vital elements and the activities of major tegumental enzymes, leading to gradual loss of physical vitality and subsequent death of *R. echinobothrida*.

Rotenone is a known bioactive compound of *M. pachycarpa*. To examine its potential anthelmintic property as a component of *M. pachycarpa*, the cestodes were incubated with varying concentrations, viz 0.5, 1, 2, 5, 10 and 20 mg/ml. Rotenone was found to cause similar motility and mortality effects, topographical damages, structural alterations, and reduction of the trace metals and activities of the tegumental enzymes in *R. echinobothrida*. The general anthelmintic effects with respect to lethal efficacy, structural damages and changes in the vital chemical constituents were found to conform to those of the extracts of *M. pachycarpa*, which suggests that rotenone is an active chemical compound of *M. pachycarpa* root bark responsible for the anticestodal activity of the plant.

It can be inferred from the observations that the extracts of both these plants apparently contain anthelmintic components that are highly cestocidals, and the type of such components ostensibly differs between the two plants, as reflected by the nature of structural changes they produced. The *A. oxyphylla* stem bark also appeared
to have significant antinematodal activity, while *M. pachycarpa* root bark failed to indicate any effect on *A. galli*. Species of *Acacia* are generally known to be rich sources of saponins and tannins, which are recognized as antinematodal principles, thus, these compounds in *A. oxyphylla* may be responsible for anthelmintic activity against *A. galli*. Specifically, only the ethanol and methanol extracts of *A. oxyphylla* were effective on the nematode and it is highly probable that the plant extracts may act preferentially as vermifugals on nematodes. Moreover, the two plant extracts appeared to enter the parasite by diffusing through the tegument in cestode and cuticle in nematode, and primarily act by damaging these interfaces. Once inside the body, they caused extensive impairment in the vital organs of the worms to bring about paralysis and death. Therefore, results from the present investigation revealed that there is scientific validity to the usage of *A. oxyphylla* stem bark and *M. pachycarpa* root bark as anthelmintics; however, the active components from the two plants need further investigation in view of the precise chemical nature, effective dose, safety and mode of action.