INTRODUCTION

Endoparasitic helminths are a group of eukaryotic macroparasitic worms belonging to the phyla Platyhelminthes and Nemathelminthes/Aschelminthes that comprised of three types of worms, cestode (tapeworms), trematode (flatworms) and nematode (roundworms). They are the most common infectious agents of humans in tropical countries and produce a global burden of disease that exceeds malaria and tuberculosis (Kabatereine et al. 2011). Helminth parasites are also responsible for a significant economic loss in livestock and crop industries around the world (De Silva et al. 2003; Hotez et al. 2008; Brooker et al. 2010). “This Wormy World” by Norman Stoll in 1947 highlighted the unbearable burden of helminth infections and since then several global efforts have been made to address the health effects of human parasitism by helminths (Stoll 1947). Over the last 60 years or so several estimates have confirmed Stoll’s initial observation that hundreds of millions of people harbor parasitic worms. Current estimates suggest that over half of the world population is infected with intestinal helminths and most of these infected people are living in remote rural areas in the developing countries (Horton 2003). Epidemiological studies have shown that among the three major classes of helminths, the global prevalence of helminthiasis caused by nematode alone constitute more than 1 billion, trematode more than 250 million and cestode about 0.4 million. Since time immemorial, helminths have plagued human life and till today they are recognized as a major agent of human infection, decreased livestock and crop production causing significant economic losses throughout tropical countries of the globe (Perry et al. 2002; Rana and Bhattacharya 2013). The direct life cycles and access by grazing to infective stages of the parasites, aided by intensive farming practices, make herbivores an easy target for many helminth parasites (McKellar and Jackson 2004). High prevalence of helminths infections among human could also be associated iron-deficiency anemia, seizures, portal hypertension and chronic diarrhea leading to high rate of morbidity and mortality (Savioli et al. 2002; Craig and Ito 2007). Although helminth infections can occur among all members of a population, the most vulnerable groups of almost all helminth infections are school-aged children and women of child-bearing age, including adolescent girls causing serious complications, such as malnutrition, anemia, bowel obstruction and learning disabilities (Savioli et al. 2002; Hotez et al. 2006). Helminthiasis also causes
substantial economic losses by smiting the livelihood and productivity of billions of world’s poorest people, however, traditionally they have been ranked low and much less attention has been paid in international and national agenda regarding its control measures compared to other disease like malaria, HIV/AIDS etc. and are regarded as “Neglected Tropical Diseases” (WHO 2010).

The basic and most common control strategy adopted against helminth infections lies mainly on the use of limited number of commercial drugs such as the benzimidazoles (BZ), the macrocyclic lactones, imidazothiazoles and the praziquantel (PZQ) (McKellar and Jackson 2004; Kaminsky et al. 2008). Because of the absence of vaccines, at present there is no alternative to chemical control. Therefore, control programs for helminthiasis are based upon a combination of chemotherapeutic control, grazing management, dietary management, biological control and ethno-veterinary treatment (FAO 2002). Anthelmintic compound BZ exert their anthelmintic effect by compromising the cytoskeleton formation through a selective interaction with β-tubulin and therefore, inhibit microtubule assembly in helminth parasites which subsequently make the parasites unable to transport secretory granules within the cell cytoplasm; resulting cell lysis (Lacey 1990). Other commercial anthelmintics like levamisole, pyrantel and morantel act as nicotinic receptor agonists and elicit spastic muscle paralysis by the prolonged activation of the excitatory nicotinic acetylcholine receptors on muscles of body wall of parasites (Martin et al. 2005; Williamson et al. 2009). Praziquantel is an important and effective anthelmintic drug registered for human and veterinary use since the early 1980s, has been found to induce increase motor activity followed by muscular contraction leading to spastic paralysis in helminth parasites at its lower therapeutic concentrations. While at higher concentrations PZQ caused tegumental damages, increases membrane permeability to monovalent and divalent cations, particularly to Ca\(^{2+}\) causing disruption in membrane calcium homeostasis (Kohn et al. 2001). The most prominent targets among them include the mitochondrial enzyme complex-I and II, cathepsin B, voltage gated Ca\(^{2+}\) ion channels, receptors such as acetylcholine and DAF-2, β-tubulin, FMRFamide-like signaling pathway (Rana and Bhattacharya 2013). Inspite of development of effective and potential anthelmintic drugs, the control of helminthiasis is one among the major health problems of people living in tropical countries because of the fact that the number of drugs available to treat
helminth infections is very limited, and most of the helminths developed resistance against prescribed doses of these synthetic drugs.

Anthelmintic resistance is a serious problem not only for livestock community but also for helminthiases of human being. High prevalence of anthelmintic resistance has now been reported from different parts of the globe for gastrointestinal helminths of livestock community (Waller 1997). Even multiple drug resistance is not uncommon in helminths of veterinary importance. The possibility of emerging drug resistance in human helminths, however do not provide conclusive evidence for the increase of innately tolerant strains or the appearance of newly mutated strain, while the anthelmintic resistance in livestock is now a well-established fact with several contributing factors which have been identified (Geerts and Gryseels 2000). High treatment frequency, single-drug regiments or frequent use of the same anthelmintic, prophylactic mass treatments, under dosing etc. generally are considered to be some of the important factors for development of drug resistance that might allow the survival of heterozygous resistant worms (Barton 1983; Smith 1990; Geerts and Gryseels 2001; Vercruysse et al. 2011a,b). Molecular studies have confirmed changes in genes or gene expression in response to drugs, change in the molecular target or change in metabolism of the parasites that enable the organism to survive treatment and might reflect evolution in a toxic environment in which drug resistance leads to ‘survival of the fittest’ (Wolstenholme et al. 2004; James et al. 2009). Therefore, because of this limited availability, in-effectiveness as well as widespread development of anthelmintic resistance, scientists are now looking for new drugs based on traditional knowledge and traditionally used medicinal plants as an alternative remedies.

The plant kingdom is known to provide a rich source of botanicals and has been used since time immemorial as a popular curative agent against various diseases of men and animals (Taylor et al. 2001). The use of plant and plant parts for the prevention and treatment of several diseases has also been documented in ancient Indian, Chinese and African civilizations. For much of our past history, forages, plant parts or extracts have been used to combat worm infections, and in many parts of the world natural products are still in use as herbal remedies (Akerele 1990; Hammond et al. 1997; Mali and Mehta 2008). Thus, plant/herbal based medicines are gaining a lot of attention and forming an integral part of the primary health care system. Reports from around the world include an exhaustive list of plants that have
been found to possess significant activity against helminth parasites. Several of such studies based on information on traditional use of herbal formulations for their putative anthelmintic properties, while in others the active ingredients responsible for the activity have been identified and characterized to establish their mode of action (Roy and Tandon 1996, 1999; Tandon et al. 1997; Tandon and Das 2007; Roy and Swargiary 2009).

The North-eastern (NE) region of India, located between 87°32’E to 97°52’E latitude and 21°34’N to 29°50’N latitude is known for its vast genetic resources all over the world and is the bio-geographical gateway to India having two-biodiversity hotspots (Mittermeier et al. 2003). The native tribes of the region have a rich tradition of using several plants in their own traditional healing system. *Alpinia nigra* is one such medicinal plant, vigorously used by the Tripuri tribes of the state of Tripura as a potential anthelmintic against various helminth infestations. Earlier studies carried out on *A. nigra* have confirmed the anthelmintic potential of crude extract of the plant against giant intestinal fluke *Fasciolopsis buski* (Roy and Tandon 1999). However, details of histomorphologicl and biochemical alterations in *F. buski* caused by the crude and its different fractions of the crude extract of the plant are not known. Therefore, it seems desirable to carry out detailed investigations involving crude and its different fractions responsible for anthelmintic properties and their possible mode of action on *F. buski*, a parasite of zoonotic importance in Northeast India.

Therefore, the aims and objectives of the proposed study include:

1. Solvent fractionation of the crude shoot extract of *Alpinia nigra* and their anthelmintic efficacy.
2. Comparison of the efficacy of the fractions with the reference anthelmintic drugs.
3. Morphological, histochemical and biochemical investigations on the effect of the crude extract and different fractions on tegumental and carbohydrate metabolism related enzymes, and trace elements of the parasite.