Review of Literature
2. REVIEW OF LITERATURE

Livestock, an adjunct to agriculture, plays an important role in the future growth and development of Indian economy. India has the largest livestock population which constitutes nearly 7% towards its national income where cattle and buffaloes generate 54% of energy for agricultural operations. The livestock provides regular employment to 18.4 million people. Helminth parasites are major constraint on animal productivity throughout the world. Gastrointestinal nematodes, trematodes and cestodes are ubiquitous parasites of grazing ruminants and cause decrease in survival, weight gain, milk, and wool production and reproduction performance. These losses can be particularly severe in developing countries where control measures are less readily available (Githigia et al., 2005; Poglaven and Battelli, 2006; Sumbria and Sanyal, 2009; Abdel-Ghaffar et al., 2011).

Incidence of gastrointestinal helminths is related to the agro-climatic conditions like quantity and quality of pasture, temperature, humidity and grazing behavior of the host (Pal and Qayyum, 1993). The serious pathological influences, epizootiological momentousness, sustainable economic losses, biochemical disorders and haematological disturbances are the prominent reasons that have earnestly impelled veterinary parasitologists to implement uncountable studies on the helminth parasites (Kareem Khoshnow Hamad, 2012).
Paramphistomosis is a disease caused by digenean trematodes belong to the family Paramphistomatidae. Adult paramphistomes parasitize in the rumen and reticulum of sheep, goats, cattle and buffaloes. Their early stages are in small intestine and then migrate through the abomasum towards the rumen (Sanabria and Romero, 2008). The harm caused by parasitic infection in bovine affects production, since these parasites provoke a lower nutritious conversion, loss of body weight and decrease in milk production, which cause economic losses (Rolfe et al., 1991). Different reports on adult paramphistomosis was recorded by species wise, month wise and season wise in India and worldwide (Hassan et al., 2005; Kanyari et al., 2009). *Cotylophoron cotylophorum* is a digenetic trematode that parasitizes the rumen and reticulum of livestock. The immature parasites are responsible for destroying the mucosal walls of the alimentary tract on their way to growing into adults by the fervent tissue obliteration the clinical symptoms are manifested. Most of the adult paramphistomes had little pathogenic significance. However severe infection with immature parasites result in the mortality of host animals (Brown, 2005; Soulsby, 2006; Foster et al., 2008; Millar et al., 2012).

Helminth parasites adversely affect the absorption and utilization of proteins, minerals and vitamins as well as upset the general metabolism of the host by causing diarrhoea, anaemia and liver disorders (Anand et al., 2000). Gupta (1993) recorded the infection with immature paramphistomes in the
small intestines of immunologically incompetent hosts. Death due to immature paramphistomes is very high and may be as high as 80-90% in domesticated ruminants (Juyal et al., 2003; Ilha et al., 2005; Hassan Syed Shabih and Juyal, 2006). It was observed that a higher incidence of paramphistomosis occurred in the months of August, September and October in slaughtered cattle and in clinically ill animals in Punjab (Khan et al., 2008). Haridy et al. (2006) reported that the two most important factors influencing the incidence of paramphistomosis are the temperature and moisture. They also emphasized that during the autumn season, the temperature and moisture are favorable for the rapid propagation of the parasitic life cycle.

The most common method used to control ruminant helminthiasis is the use of chemical compounds commercially available as anthelmintic drugs that are regularly administered to animals for deworming which is considered to be simple, safe and cheaper (Jackson, 2009). Antiparasitic chemotherapeutics can be categorized as anthelmintics and ectoparasiticides (Taylor et al., 2007). Anthelmintic is a destructive agent to parasites, which has been classified as antinematicidal, antitremericidal, and anticesticidal. Martinez and Cruz (2009) opined that there are several disadvantages in the use of synthetic drugs such as their adverse effect against beneficial microorganisms in soil once they are eliminated with the faeces. On the other hand, some anthelmintic compounds can remain as contaminants in animal
products destined for human consumption i.e., meat, milk, etc. (FAO, 2002). One of the main concerns in the use of anthelmintic drugs for controlling ruminant parasites is the development of anthelmintic resistance in the parasites that decreases the efficacy of the drugs (Sutherland and Leathwick, 2011; Torres-Acosta et al., 2012) and threatens economical sustainability of sheep production (Sargison, 2011). The anthelmintic resistance can reach enormous proportions when parasites develop mutations in their genome against different groups of anthelmintic drugs. Such phenomenon is known as “Multiple anthelmintic resistance” and it is a real threat to the inefficacy of commercially available anthelmintics (Taylor et al., 2009; Saeed et al., 2010).

Successful control of intestinal helminths remains the major conundrum in livestock agriculture throughout the world. Gilleard (2006) and Besier (2007) stated that the tremendous developments in the discovery and understanding of the pharmacology of anthelmintic drugs have not ameliorated the global crisis of helminth infestations and rapid evolution of anthelmintic resistance virtually to all types of chemotherapeutic drugs has become the paramount threat to animal industry. Moreover, global appreciation of organic farming also constrains further dissemination of chemotherapeutics, as synthetic anthelmintics are proven to have detrimental effects on non-target organisms and the environment in general (McKellar, 1997). Such situations have motivated workers around the world to look for alternatives to control the parasitic infections. Searching for plant
bio-active compounds with medical properties against parasites has gained great interest in order to at least partially replace the use of chemical drugs (Pedro Mendoza de Gives, 2012).

Medicinal plants are resources of new drugs and have served through ages, as a constant source of medicaments for the treatment of a variety of diseases and are known to provide a rich source of botanical anthelmintics. A wide range of plants and their products around the world are being explored to look for their possible anthelmintic effects on cestodes, trematodes (Abdel-Ghaffar et al., 2011) and nematodes (Datsu Kalip et al., 2011). Rahmatullah et al. (2010) reported that the medicinal plants used by folk medicinal practitioners in Balidha village of Jessore District, Bangladesh around nineteen plants were used for treatment of gastrointestinal disorders and seventeen plants for treatment of sexual disorders. In another survey Hasan et al. (2010) reported 80 plants distributed into 45 families were used as whole plants as well as plant parts for the various ailments by the local tribal people in Pabna District, Bangladesh.

Due to the important economic impact of gastrointestinal parasites in the livestock industry around the world, most of the researches on plant extracts are being focused on searching bioactive compounds from plants against many important groups of parasites. Plants have the anthelmintic activity mainly due to their phytoconstituents especially due to secondary metabolites. However, the mechanism of anthelmintic action of the secondary
metabolites is not yet properly understood. The usage of plant anthelmintics to combat gastrointestinal helminths of small ruminants is well documented by several parasitologists (Hammond et al., 1997; Schillhorn van Veen, 1997; Akhtar et al., 2000; Tagboto and Towson, 2001; Githori et al., 2006; Veerakumari and Navaneetha Lakshmi, 2006; Veerakumari et al., 2012; Priya et al., 2013; Piyush et al., 2013; Chhabra et al., 2014; Manoj Dhanraj and Veerakumari, 2014; Veerakumari et al., 2014; Veerakumari, 2015).

Aqueous and various solvent extracts viz. hexane, chloroform, ethylacetate, alcoholic extracts, pastes, essential oils, extracted from leaves, stem, fruits, flowers and roots or whole plants were used to test their anthelmintic efficacy in vitro or in vivo. Alcoholic extracts of Allium sativum possess antiparasitic activity against Heterakis gallinae, Ascardia galli, Gigantocotyle explanatum and Cotylophoron cotylophorum (Sutton and Haik, 1999; Singh and Nagaich, 2000; Singh et al., 2008; Nahla et al., 2012). Asha et al. (2001) reported that Ocimum sanctum as a potent anthelmintic against Caenorhabditis elegans in vitro. Legume Sericea lespedea possess remarkable anthelmintic efficacy on Haemonchus contortus (Min et al., 2004; Shaik et al., 2004; Lange et al., 2006). The anthelmintic efficacy of Citrullus colocynthis found to be positive on H. contortus, which caused reduction in egg count (Ullah et al., 2013). In the present study the anthelmintic efficacy of Adhatoda vasica and Piper betle was investigated against the paramphistome Cotylophoron cotylophorum.
*Adhatoda vasica* commonly known as Vasaka or Arusha is a well-known herb in indigenous systems of medicine belongs to the family Acanthaceae. *A. vasica* is a perennial evergreen and highly branched shrub with unpleasant smell and bitter taste; the plant lives for multiple seasons and retains its leaves throughout the year. It is a shrub 1.0 m to 2.5 m in height, with opposite ascending branches. It grows all over India and in the lower Himalayan ranges. Beside India, it is found in Myanmar, Sri Lanka, Burma and Malaysia. *A. vasica* is used to treat for bronchitis, tuberculosis and other lung disorders (Sampath Kumar *et al*., 2010; Sunita and Dhananjay Singh, 2010). Studies also have indicated that *A. vasica* possess anti-inflammatory, analgesic, antioxidant, hepatoprotective, sedative, antispasmodic and anthelmintic properties (Mulla *et al*., 2010). *A. vasica* leaves showed significant antifungal activity (Khare, 2007), wound healing effect (Vinothapooshan and Sundar, 2010), antihistaminic effect, moderate hypotensive activity, thrombopoeitic effect (Mahajan *et al*., 2010), antidiabetic (Bhatt *et al*., 2011), antiulcer (Vinothapooshan and Sundar, 2011), antimicrobial (Sheeba and Mohan, 2012) and antibacterial activities (Kavitha *et al*., 2012).

Preliminary phytochemical study of *A. vasica* revealed the presence of alkaloids, glycoside, tannins, saponin, steroids, tri-terpenoid and flavonoids (Arabind Kumar *et al*., 2013). The leaves contain two major alkaloids called vasicine and vasicinone and also rich in vitamin C, carotene and essential oil.
The roots are known to contain vasicinolone, vasicol, peganine, sitosterol, β-glucoside-galactose and deoxyvasicine and 2'-hydroxy-4-glucosyl-oxychalcone. The flowers of *A. vasica* contain b-sitosterol-D-glucoside, kaempferol, its glycosides and quercetin; minor alkaloids include adhatonine, vasinol (Khare, 2007; Ahmad and Garg, 2009; Mulla *et al*., 2010; Sampath Kumar *et al*., 2010; Borooah, 2011).

*Piper betle* is a glabrous climbing vine belongs to the family Piperaceae, this plant is native to central and eastern Malaysia and was taken into cultivation more than 2500 years ago throughout Malaysia and tropical Asia. In India *P. betle* is widely distributed in all the states except northern regions (Jammu and Kashmir, Haryana, Punjab, Himachal Pradesh), due to severe winter and northern west (Rajasthan, Gujarat) due to hot dry summer (Guha, 2006; Kumar *et al*., 2010). *P. betle* is commonly known as betel vine in english as paan in assamese/urdu/hindi/odia/bengali, and tambula and nagavalli in sanskrit, vetrilai in tamil (Rai *et al*., 2011; Satish *et al*., 2013).

Leaves of *P. betle* are a heart shaped with different size. The size of the leaf varies with different cultivar from 7 to 15 cm in length and 5 to 14 cm in width they are simple alternate stipulate petiolate with 0.75 to 3.8 cm, ovate oblong broadly ovate cordate or obliquely elliptic entire glabrous coriaceous 10 to 18 cm long and 5 to 10 cm broad acuminate oblique and rounded base. Leaves are yellowish green to dark green in colour with glossy upper surface having characteristic and pleasant odour. Taste of leaves ranging from sweet
to pungent due to the presence of essential oils (Lakshmi and Naidu, 2010; Vasuki et al., 2011; Periyenayagam et al., 2012).

In ayurveda P. betle leaf extract is frequently used as an adjuvant and mixed with different medicines possibly for better effects beside its independent use as medicine (Kumar, 1999). P. betle used as avata and kapha suppressant in traditional treatment and also helps in expelling out the mucus from the respiratory tract because of its hot potency (Balkrishna, 2008). The leaves, which are the most commonly used plant part of P. betle, are pungent with aromatic flavour and are widely consumed as a mouth freshener. It is carminative, stimulant, astringent and is effective against parasitic worms (Farhan Fazal et al., 2014).

Rekha et al. (2014) stated that the leaves of P. betle contain protein, tannins, minerals like calcium, phosphorus, iron, iodine and potassium, and also contains vitamin B, vitamin C and vitamin A. Arambewela et al. (2005), Ghosh and Bhattacharya (2005), Kanjwani and Marathe (2008) reported the presence of phenol and terpenoids include 1, 8-cineole, cadinene, camphene, caryophyllene, limonene, pinene, chavicol, allyl pyrocathecol, carvacrol, safrole, piperitol, eugenol, isoeugenyl acetate, isoeugenol, chavicol and chavibetol in P. betle. Recently number of investigations accounted that the P. betle leaves contains starch, diastases, sugars and an essential oil composing of safrole, allyl pyrocathecol monoacetate, eugenol, terpinen-4-ol, eugenyl acetate, etc. as the major components (Fong, 2009; Chaurasia et al.,
2010; Dwivedi et al., 2010; Arambewela et al., 2011; Chandra et al., 2011; Dwivedi and Mehta, 2011; Rai et al., 2011; Sugumaran et al., 2011; Misra et al., 2014). *P. betle* leaves has a significant antimicrobial activity (Adeltrudes and Marina, 2010; Intzar et al., 2010; Kumar et al., 2010; Niraj et al., 2010; Arani Datta et al., 2011; Kaveti and Tan, 2011; Devjani and Barkha Shah, 2011; Jahir Alam Khan and Naveen Kumar, 2011; Mahfuzul Hoque et al., 2011; Patturajan Rajeshbabu et al., 2011; Jesonbabu et al., 2012; Tarun Agarwal et al., 2012).

Leaves of *P. betle* possess antifertility (Sharma et al., 2007), anti-ulcerative property, hepatic protective activity, antioxidant and mucus protecting properties (Bhattacharya et al., 2007). *P. betle* leaves extracts exhibit anti-allergic effects (Mali Wiroteangthong et al., 2007) anti-leishmanial (Pragya Misra et al., 2009) anti-filarial (Meghana et al., 2009), anti-halitosis (Niranjan Ramji et al., 2002), antifungal (Gupta et al., 2009; Ali et al., 2010; Intzar et al., 2010), antioxidant, antihistaminic and anti-inflammatory effect (Choudhury and Kale, 2002; Saravanan et al., 2002; Dasgupta and De, 2004; Panuwat Suppakul et al., 2006; Rathee et al., 2006; Pitchaon Maisuthisakul, 2007; Pushpavalli et al., 2008; Tripathi, 2008; Pushpavalli et al., 2009; Pin et al., 2010; Rahul Hajare et al., 2011). An insecticidal activity of essential oil from *P. betle* has reported by Cristina et al. (2009). Anti-larvicidal activity of *P. betle* was observed by Arambewela et al. (2011) against *Chrysomya megacephala*. Gastro protective


Different plant compounds or active principles have different targets to exert antihelmintic effect on flukes. There may be inhibition of enzymes, making complexes with proteins, polysaccharides, forming channels of ions etc. Anthelmintics might disturb the usual biochemical and physiological processes by depriving nutrition, changing structure and disturbing neuromuscular transmission in helminths (Kohler, 2001; Mottier \textit{et al.}, 2006). Mali and Mehta (2008) illustrated the need for phytochemical studies to
standardize the anthelmintic activity of plant extracts and formulate best alternative herbal preparations to replace synthetic drugs which are currently in use.

The mode of working mechanism of phytoconstituents as anthelmintic have been explained by various researchers (Patel et al., 2010, John et al., 2009, Roy et al., 2010, Borba et al., 2010) the possible mode of action of phytoconstituents is shown Fig. 1.

The most common effect of anthelmintic drugs against parasitic infections is paralysis of the parasite musculature by inhibiting the neuromuscular transmission (Mohammed et al., 2005; Behnke et al., 2008). A semisynthetic agent ivermectin, obtained from an actinomycete, paralyses the parasites by opening chloride channels and increasing chloride conductance (Rang and Dale, 2003). Piperazine can be used to treat infections with the common round worm and the threadworm. Chandrashekhar et al. (2008) reported disruption of the mucopolysaccharide membrane of worms will expose the outer layer restricting their movement which finally may cause paralysis and ultimately death of parasite. Roy et al. (2010) reported that alkaloids act on the central nervous system and impair the motility of parasites.

Mansour (2002) stated the motility and survival of helminths are controlled by proper neuromuscular coordination system and any interference
Fig. 1 Mode of Action of various phytoconstituents on helminth parasites
to this system could lead to the paralysis and expulsion from the host body or even death of the parasite. All broad spectrum anthelmintic drugs regardless of their mode of action drastically reduce parasite acetylcholinesterase (AChE), thus making it as a potential target (Rapson et al., 1986). Motility studies directly correlate with the neuromuscular physiology of the trematodes (Kumar et al., 1995). Inhibition of motility and AChE of *C. cotylophorum* by various plant extracts *viz.* *Allium sativum*, *Punica granatum*, *Syzygium aromaticum*, *Terminalia chebula* and *Prosopis cineraria* are on record (Veerakumari et al., 2012; Manoj Dhanraj and Veerakumari, 2014; Lokesh and Veerakumari, 2015; Manigandan and Veerakumari, 2015).

In general, the surface tegument acts as the vital organ of parasites, performing various functions like absorption of food materials, protection and osmoregulation and the suckers which are modification of tegument, offers organ of anchorage. Disruption in normal ultra-structure of tegument is necessary to develop any novel drugs which may able to damage the parasite and caused mortality, targeting tegument of the parasites. (Manger, 1991; Veerakumari and Munuswamy, 1999; Veerakumari and Paranthaman, 2004; Veerakumari et al., 2012; Radwan et al., 2012; Swarnakar et al., 2014). The tegument is not only a protective shield for the parasite but performs other important functions at the interface between the parasite and its host (Skelly and Shoemaker, 1996). Mottier et al. (2006) reported that the
synthetic anthelmintics might disturb the biochemical and physiological processes by changing structure and disturbing neuromuscular aspects in helminths.

TEM investigations by Lorsuwannarat et al. (2013) on the effect of plumbagin against *Faciola gigantica* revealed erosion and lesion on the tegument. Naguleswaran *et al.* (2006) reported a high degree of vacuolated cytoplasm, large numbers of lipid droplets, electron dense and rounded mitochondria, and, in some areas, separation of laminated layer from the tegumental tissue in *Echinococcus multilocularis* and *E. granulosus* treated with genistein. The crude extract of *Flemingia vastita* caused disorganization of cuticle and body musculature in treated *Ascaris suum* (Yadav *et al.*, 1992) and destruction in tegument and distortion of muscles, vacuolisation in muscles of sucker indifferent helminth parasites (Tandon *et al.*, 1997). The extract of *Lasimachia ramosa* induced destructive surface alteration in *Ascaris suum* and *Fasciola buski* (Challam *et al.*, 2010). Swarnakar *et al.* (2014) has demonstrated anthelmintic activity of *Trigonella foenum-graecum* against the amphistome *Gastrothylax crumenifer* which initiated blebbing and detachment of the tegument. Chandrashekhar *et al.* (2008) reported that any damage to the mucopolysaccharide membrane of parasites will expose the outer layer restricting their movement which finally may cause paralysis and ultimately death of the parasites.
The universal feature of endoparasitic organism is their dependence upon anaerobic carbohydrate metabolism to obtain energy and glycogen is considered as the chief energy reserve. Evidence for the functioning of different metabolic pathways in parasites has been adduced mainly by the demonstration of the enzymatic steps or the identification of the intermediates of the pathway. The wide distribution of many glycolytic enzymes and the demonstration of phosphorylated glycolytic intermediates within the bodies of numerous parasites clearly indicate the operation of typical glycolytic sequences until phosphoenol pyruvate or pyruvate is reached (Saxena, 2006).

Glycolysis, the main energy-generating pathway, with lactate as an end product in nematode parasites (Omar et al., 1996), seems to be a promising target for new drugs against parasites, because this pathway plays an essential role in ATP supply (Lakhdar-Ghazal et al., 2002). During anaerobic condition phosphoenolpyruvate (PEP) is obtained from glycolysis is converted to pyruvate by the action of pyruvate kinase (PK), which is further reduced to lactate, by lactate dehydrogenase (LDH). When the activity of PK is low, carbon dioxide fixation to PEP take place by the action of phosphoenolpyruvate carboxykinase (PEPCK) which is a vital step in energy metabolism. PK is functionally connected with PEPCK. Both enzymes having a competition to bind the common substrate PEP to work an aerobic and anaerobic metabolic pathway. Several investigations were made to elucidate the influence of anthelmintics on the carbohydrate metabolism of helminth
parasites (Bueding and Fisher, 1970; Schulman et al., 1982; Donahue et al., 1983). In general, benzimidazoles are known to disturb the energy metabolism of parasites (Zia and Nizami, 1998; Venkatesan, 1998). Effect of *Acacia concinna* and *Punica granatum* on key enzymes involved in carbohydrate metabolism of *C. Cotylophorum* was demonstrated well by Priya and Veerakumari (2011) and Veerakumari et al. (2014).

The main role of PEPCK in helminths appears to be the opposite of their vertebrate hosts; in the former it is involved in the degradation of the glucose molecule, whilst in the latter its main role is in gluconeogenesis. Because of the differing primary functions of PEPCK in helminths and their hosts, this enzyme might be inhibited selectively and thus provide an avenue for anthelmintic attack (Reynolds, 1980). The inhibition of PEPCK by cambendazole in *Moniezia expansa* has been ascribed to diversion of metabolic activity towards lactate production (Rahman and Bryant, 1977). Navaneetha and Veerakumari (2009) reported the inhibitory effect on the PK and PEPCK activities in *Haemonchus contortus* treated with *Allium sativum*. Artmether, a derivative of Artemesia and well known for its antimalarial properties, was shown to exert a potent inhibitory action on PK (Xiao et al., 1998) and LDH in *Schistosoma japonicum* (Xiao et al., 1999).

An important step in the anaerobic metabolic processes is the reduction of oxaloacetate catalyzed by the cytoplasmic malate dehydrogenase (MDH). The reduction of malate to succinate occurs in two reactions in the
reverse part of the Krebs cycle and the reduction of fumarate is the essential NADH consuming reaction to maintain redox balance. The OAA formed is reduced by NADH to malate thereby regenerating glycolytic NAD. The cytoplasmic malate is not excreted whereas it is transported to mitochondria for further anaerobic metabolism. Mitochondria utilize malate as a main substrate for two other linked pathways called malate dismutation: malate is reduced in a series of reactions and oxidized in another series of reactions to maintain redox balance (Saz and Lescure, 1969). Barrett (1981), stated that the cytoplasmic malate dehydrogenase exist in higher activity than its mitochondrial counterpart. Oztop et al. (1999) reported that the albenzazole and niclosamide altered the MDH and LDH activities of Trichuris saginata. Similar inhibitory effect of Acacia concinna on the cMDH and mMDH activity of C. cotylophorum was reported by Priya and Veerakumari (2011). In the reduction route of the malate dismutation reaction FR catalyses the reduction of fumarate to succinate. Barrowman et al. (1984) recorded the inhibition on FR by various benzimidazole compounds.

Fumarate is reduced to succinate using NADH as reducing equivalent and succinate formation is the final step of the glycolytic pathway (Maule and Marks, 2006). Sakai et al. (2012) observed that nafuredin inhibited NADH- fumarate reductase of nematode Ascaris suum. In PEP-Succinate pathway succinate formation is the final step. In helminths there is a partial reverse TCA cycle from succinate to oxaloacetate but in the reverse direction, in
mammals succinate is being oxidized to fumarate. Succinate is a major end product in parasitic helminths which can be further metabolized to propionate and short chain fatty acids. The conversion of succinate to propionate is not an ATP requiring processes and is formed by a reversal of the pathway that occurs in mammals during the formation of succinyl-CoA from succinate.

Succinate and pyruvate, the products of malate dismutation are utilized by helminths as precursors for the anaerobic formation of the volatile fatty acid end products (Saz and Weil, 1960; 1962). Varying degree of inhibition of FR and SDH activity were observed in *H. contortus* treated with tetramisole and rafoxanide *in vitro* (Kaur and Sood, 1983). The inhibitory effect of ivermectin, mebendazole, on SDH activity and tribendazole on FR activity of *Trichinella spiralis* has been reported by Rodriguez *et al.* (1985). Veerakumari (1996) reported that praziquantel (PZQ), levamizole (LVM), and benzimidazoles (BZM) significantly inhibited the FR and SDH activity of *C. cotylophorum*.

Glucose and glycogen are the major energy sources in helminth parasites (Barrett, 1981; Roy *et al.*, 2012). Ahmad and Nizami (1987) reported that mebendazole induced inhibition of glucose uptake and glycogen depletion in *Avitellina lahorea in vitro*. Tandon *et al.* (2003) observed significant depletion of glycogen in *Raillietina echinobothrida* treated with *Flemingia vestita*. The mechanism of action of albendazole is by blocking glucose uptake in larval and adult stages of susceptible parasites, and also
depleting their glycogen reserves, thus decreasing ATP formation (Martin, 1997). Jain et al. (2011) reported in that phytoconstituents, jointly or individually block glucose uptake in parasites. There are reports that pectin (extracted from various fruits) administration decreased the absorption of glucose which may also contribute partly to the hypoglycemic action (Holt et al., 1976; Kanter et al., 1980; Schwartz et al., 1980; Vaaler et al., 1980; Gomathy et al., 1990). A methanolic extract of cumin seeds reduced the blood glucose and inhibited glycosylated hemoglobin, creatinine, blood urea nitrogen and improved serum insulin and glycogen content in alloxan and streptozotocin (STZ) diabetic rats (Dhandapani et al., 2002; Jagtap and Patil, 2010).

Tegumental enzymes play important role in maintaining the tissue homeostasis within the parasite. Alkaline phosphatase take part in active transport through cellular membranes and acid phosphatase deals with intracellular digestion (Lumsden et al., 1968 and 1975; Pappas and Read, 1975; Read, 1996), phosphorylation of nutrients transported, secreted and excreted (Maki and Ianagisawa, 1980). Anthelmintics may alter the acid phosphatase and alkaline phosphatase and modify the normal metabolism of the absorptive surfaces in parasites and react with the substances in the external milieu (Pappas and Read, 1975). Calotropis procera showed inhibitory effect on tegumental enzymes of Gastrothylax indicus (Rama and

Acetylcholine (ACh) is an important neurotransmitter in both free-living and parasitic nematodes and also associated with the neuromuscular system. The classical role of acetylcholinesterase (AChE) is to terminate transmission of neuronal impulses by rapid hydrolysis of ACh. The presence of choline and choline acetyl transferase has also been revealed in many parasites (Sukhdeo *et al*., 1986). Mansour (2002) reported that any interference to neuromuscular coordination system could lead to the paralysis and expulsion from the host body or even death of the parasites. In trematodes, AChE is found to be associated with surface teguments and internal sub-cellular structures (Roy *et al*., 2012). Martin (1997) revealed that the AChE has been targeted by many drugs and poisons, both natural and synthetic.

A large number of drugs and photochemicals have been investigated to reveal their interference on AChE activity (Geary *et al*., 1992; Veerakumari and Priya, 2006; Swargiary and Roy, 2011). Swargiary and Verma (2015) reported in their docking study that mebendazole showed the highest binding affinity with AChE than albendazole and praziquantel. Similar studies were carried out by Gupta *et al*. (1991) on acetylcholinesterase activity of *Ascaridia galli* and its kinetic properties revealed inhibition of AChE activity on exposure of the worm to commercial anthelmintics drugs piperazine,
adipate and albendazole. Aqueous extract of *Mentha longifolia* has rich source of various natural AChE inhibitors (Chandra Shekhar and Suresh Kumar, 2014 and Vladimir *et al.*, 2014).

All living organisms need to maintain an adequate intracellular redox environment. Glutathione has vital functions as thiol redox buffer and cofactor of antioxidant and detoxification enzymes. Glutathione-s-transferases (GST) involved in protecting cells against reactive oxygen species, toxic metabolic intermediates and xenobiotics (Meister, 1983; Meister and Anderson, 1983; Meister, 1988; Becker *et al.*, 2003). GST play an important role in normal cellular metabolism as well as in the detoxification and may repair host induced damages (Mannervik, 1985; Ketterer *et al.*, 1989; Mitchell, 1989; Marrs, 1996; Saxena *et al.*, 1996).

GST has been found in helminths, such as trematodes and nematodes (Rao *et al.*, 2000). Farahnak and Jefferies (2005) reported the level of GST is approximately 4% of the total soluble protein in helminth parasites. The significant role of GSTs in lipid peroxidation was observed in cestode and digenean trematodes by many workers (Brophy and Barrett, 1990; O'Leary and Tracy, 1991; Brophy and Pritchard, 1992a). Liebau *et al.* (1996) characterized the GST in *Echinococcus multilocularis*. The activity of GST has been reported in *Faciola gigantica* (Singh and Irshadullah, 2003), *F. hepatica* (Howell *et al.*, 1988), *E. granulosus* protoscoleces (Seyyedi *et al.*, 2005), *T. solium* (Vibanco-Perez *et al.*, 1999, 2002) and *Schistosoma mansoni*
(Mei and LoVerde, 1997). Feng et al. (1995) suggested that the inhibition of GST activity with mebandazole might damage the defense mechanism of the parasites. The biological role of GST in parasite is important for understanding the host-parasite relationship and any change in their functions could have therapeutic implications. Because of its role in protecting the cell against the immune-mediated lipid peroxidation, GST is considered as one of the vital targets for anthelmintic drugs (Singh and Irshadullah, 2003).

The preliminary phytochemical screening of the plant extracts is mandatory to identify the presence of tannins, flavonoids, alkaloids, saponins, sterols and terpenes. Many countries have focused on screening of plant extracts with anthelmintic properties from their native flora with an enormous potential for the control of animal parasitic nematodes with promising results. Some countries i.e. Brazil, India, South Africa, China and others possesses an extraordinary richness in their medicinal flora and they have currently developed an important industry from plant extracts supported by advanced technology. It is imperative to decrease the reliance on these chemotherapeutic drugs for parasite control, not only because of resistance, but also because of growing concerns about the adverse consequences of these anti parasitic drugs on the ecosystem and biodiversity (Veerakumari, 2015). This danger has given impetus to the search for new drugs, with attention focusing on the search for plant products and the application of plant products as alternative methods of control. In this regard Iqbal et al. (2001),
Githiori *et al.* (2006) and Veerakumari (2015) have developed a solid package of information about plants with encouraging results in the control of sheep parasites.

Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators (Niezen *et al.*, 1995; Waller *et al.*, 1997; Athanasiadou *et al.*, 2001). The polyphenolic compounds (tannins) are shown to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite (Thompson and Geary, 1995), and cause death. Athanasiadou *et al.* (2001) declared that anthelmintic effects of tannins may be attributed to its capacity to bind free protein available for larval nutrition and thus reducing the nutrient availability resulting in larval starvation or decrease in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation causing larval death. Borba *et al.* (2010) stated that the antioxidant effect of steroidal alkaloid and oligoglycosides is capable of reducing the nitrate generation and may interfere in local homeostasis which is essential for the development of helminths.

Trace elements are cofactors for and components of enzymes and therefore have pivotal roles in biochemical reactions that can have widespread repercussions in animal metabolism and physiology. Biological functions are often concerned with the activities of multiple enzymes. Consequently, deficiency has considerable and varied scope for limiting a range of
immunological responses either directly or via other physiological systems (McDonald et al., 1988; Underwood and Suttle, 1999). Several attempts have been made to identify and quantify the inorganic contents in larval forms of parasitic cestodes (Singh et al., 1978; Jakutowicz and Korpaczewska, 1979). Mertz (1981) opined that for each element, there is a range of safe and adequate exposures permitting optimal biological function, below which range there is deficiency. Every trace element is potentially toxic when this range is exceeded. McDonald et al. (1988) suggested the macro elements such as calcium, phosphorus, magnesium, sulphur, sodium and potassium can be important constituents of tissues, they involved in maintenance of acid–base balance, the osmotic control of the body and oxygen transport. However, most of the major elements have a third role, as do many trace elements such as iron, zinc, copper, molybdenum, selenium, cobalt and iodine, in the catalysis of enzyme systems, either as integral components of metallo-enzymes or as activators within enzyme system.

The importance of inorganic substances to adult cestodes is demonstrated by experimental studies involving mineral deficiencies in the host's diet (Chand, 1969; Deo and Srivastava, 1962; Von Brand, 1966; Mathur and Pande, 1969; Nadakal et al., 1975). More reports have highlighted the possibility that trace element deficiencies may affect nematode survival and reproduction (McClure et al., 1999; Coop and Kyriazakis, 2001; Koski and Scott, 2003). Mathur and Pande (1969) observed that calcium deficiency diet
of host leads to dwarfing of *Raillietina cesticillus*. Singh *et al.* (1978) stated the phosphorus in the mature region of *Thysaniezia giardi* is higher than gravid region. Varying mineral levels in different regions of the parasites were reported by several researchers (Goodchild *et al.*, 1962; Singh *et al.*, 1978). The immature region of the tapeworm contained more amount of potassium which forms the major base of the body cells and also involved in osmotic pressure regulation and acid-base balance (Nadakal and Vijayakumaran Nair, 1981). Copper and zinc are co-factors for the normal functioning of many vital enzymes observed in parasites (Symyth, 1969; Enigk *et al.*, 1976; Vasilev *et al.*, 1976). Shi *et al.* (1995) reported that zinc deficiency affects the growth, survival and reproduction in *Heligmosomoides polygyrus*. Nadakal and Vijayakumaran Nair (1981) noted the higher concentration of Cu and Zn in the immature region of *Raillietina tetragona*. The significance of zinc for the reproduction of the parasite (Chowdhury and Singh, 1989) and zinc-dependent enzymes have been reported by (Marco and Nieto, 1991) in *Echinococcus*. Olds *et al.* (1990) reported that boron is required for survival of nematodes.

*In vivo* observations on haematological and biochemical profiles of the animals can offer more insights into the safety and protective efficacy of the plant extracts. Verma *et al.* (2006) reported the faecal egg count reduction test is suitable for the evaluation of all types of anthelmintics. Furthermore, haematological parameters and biochemical profiles are important diagnostic
tools for assessing the level of infections and therefore may exhibit quantitative changes between pre and post treatments (Priya et al., 2013). Navaneetha et al. (2011) observed the enhanced haematobiochemical profile in sheep after treated with *Allium sativum* against strongyles. Similar observations on improved haematological and biochemical values following treatment with anthelmintics have been observed in goats, cattle and buffaloes (Moreno et al., 1997; Shaikh et al., 2006; Verma et al., 2006).

The above review gives the clear idea about the increasing interest towards ethno-veterinary practices across the world especially the use of medicinal plants in treating various ailments exclusively for helminth infections. Based on the current scenario this study was designed to elucidate the anthelmintic efficacy of *Adhatoda vasica* and *Piper betle* against the paramphistome *C. cotylophorum* in vitro and in vivo.