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EFFECT OF ETHANOL EXTRACT OF *TRACHYSPERMUM AMMI* ON PYRUVATE KINASE AND PHOSPHOENOLPYRUVATE CARBOXYKINASE OF *COTYLOPHORON COTYLOPHORUM*

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ABSTRACT

Parasitic helminths represent one of the most pervasive challenges to livestock. Paramphistomosis commonly affects cattle and sheep, and is caused by various species of the paramphistomidae. Anthelmintic drugs are used to treat helminth infection. The high incidence of resistance of helminth parasites to anthelmintic drugs in addition to the relative toxicity and side effects of many of these drugs urge the necessity of finding alternative eco-friendly agents against helminths. This applies to plant-based anthelmintics that have been used to kill and expel the parasites from gastrointestinal tract. The parasites depend for their energy almost entirely on carbohydrate metabolism which is essentially anaerobic and involves the glycolytic and part of the reversed tricarboxylic acid (TCA) cycle. In parasitic helminth's energy metabolism, phosphoenol pyruvate (PEP) is a branch point which is either converted to pyruvate by pyruvate kinase (PK) or to oxaloacetate by phosphoenolpyruvate carboxy kinase (PEPCK) results in ATP synthesis. Hence the efficacy of ethanol extract of *Trachyspermum ammi* (*Ta*EE) was assessed based on its effect on PK and PEPCK against *Cotylophoron cotylophorum*. The parasites were incubated in five different sub-lethal concentrations of *Ta*EE for 2, 4 and 8h. PK and PEPCK was assessed using standard procedure. The enzyme activity was expressed in terms of protein. The data obtained were analyzed statistically. Maximum level of inhibition in PK and PEPCK was observed after 8h of incubation in *Ta*EE. Inhibition of PK and PEPCK was dose and time dependent. Inhibition of PK and PEPCK in drug-treated flukes blocks the carbon flow from PEP to the end product of anaerobic metabolism. It eventually leads to reduction in the synthesis of ATP in drug-treated flukes. Decreased production of ATP results in the death of the parasites.

INTRODUCTION:

Gastrointestinal parasitic infections are a worldwide problem for small domestic ruminants like sheep, goat and cattles. Paramphistomosis is caused by widespread infection of the small intestines with immature paramphistomes [(1)]. Paramphistomosis has been associated with a few species of paramphistomes, viz. *Paramphistomum cervi, P. explanatum, Cotylophoron cotylophorum, Gastrothylax crumenifer* and *Fischoederius elongates* [(2)]. *Cotylophoron cotylophorum* [(3)] is more prevalent in Tamil Nadu [(4,5)], occurs in the

rumen of the sheep, goat, cattle and other domestic ruminants. Immature flukes live in the small intestine of ruminants where they attach themselves to the intestinal lining with suckers, whereas, adult flukes are found in the rumen or reticulum [(6)]. Chemotherapy is a major treatment used to control paramphistomosis. The chemotherapeutic drugs exhibit severe side effects to the animals and also the drugs are expensive and non- biodegradable. Continuous exposure of parasites to chemical anthelmintics leads to the ultimate development of resistance. Synthetic anthelmintic treatments are often impracticable in developing countries due to relatively high price of these anthelmintics [(7)]. This danger has given impetus to the search for new drugs, with attention focusing on the search for plant products and the applications of plant products as alternative methods of control.

Anthelmintics from the natural sources play a key role in the treatment of parasitic infections, which have suggested to the proposal of screening medicinal plants for their anthelmintic activity. Medicinal plants are the major source of many primary and secondary metabolities. A number of medicinal plants are used to combat parasitism, and in many parts of the world are still using to treat parasitic infections in man and animals. Plant based anthelmintics, especially, the phytochemicals have gained considerable importance due to their potential health benefits [(8)]. Many biochemical constituents of plants have been shown to possess excellent biological activites [(9)]. Plant extracts with high concentration of secondary metabolities such as saponins, tanins, flavonoids have been found to kill gastrointestinal parasites. *Trachyspermum ammi* belongs to the family Apiacease. It is commonly called Omum in Tamil. *T. ammi* is used traditionally as a carminative, stimulant, flatulence atonic dyspepsia, abdominal pains piles, abdominal tumors, asthma and amenorrhoea [(10)]. *T. ammi* has been medicinally proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive, antihypertensive, antispasmodic, antilithiasis, antidiuretic, antitussive, anthelmintic anti-inflammatory, anti-aggregatory and antifilarial activity [(11-14)]. In helminth parasites, carbohydrates are the sole energy source for the survival of the parasite. The pathway of carbohydrate metabolism is essentially anaerobic and involves the glycolytic and part of the reversed tricarboxylic acid (TCA) cycle [(15)]. The energy production shifts to anaerobic ones with lactic acid as the end product of glycolytic pathway instead of pyruvic acid. The present study proposed to elucidate the effect of ethanol extract of *Trachyspermum ammi* on pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK) the key regulatory enzymes involved in carbohydrate metabolic pathway of *Cotylophoron cotylophorum.*

MATERIALS AND METHODS

Collection of parasites and *in vitro* maintenance

Adult *C. cotylophorum* (Fig. 1) were collected from infected sheep at Perambur slaughter house, Chennai. Live flukes were transferred to a petridish, washed thoroughly in physiological saline and parasites were maintained in Hedon-Fleig solution (pH 7.0).

Preparation of Hedon-Fleig solution

Hedon-Fleig solution (pH 7.0) is a suitable medium for the *in vitro* maintenance of *C. cotylophorum* [(4)]. It is prepared by dissolving 7 g of sodium chloride, 0.1 g of calcium chloride, 0.3 g of potassium chloride, 1.5 g of sodium bicarbonate, 0.5 g of disodium hydrogen phosphate, 0.3 g of magnesium sulphate and 1 g of glucose in 1000 ml of distilled water.

Extract Preparation of Plant Material

Trachyspermum ammi seeds (Fig. 2) were purchased from local market and coarsely powdered, soaked in ethanol for 48 h. Aqueous extract was also prepared. Filtration done through Wattman filter paper no1. Distillation has been done with rotary evaporator (EQUITRON). Extract was kept in Lyodel freeze drier (DELVAC) to remove the solvent and dried up. Stock solution (10%) was serially diluted with Hedon-Fleig solution to obtain sub-lethal concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) of *Ta*EE.

Sample preparation

Control and the drug-treated flukes were rinsed in distilled water. The flukes were weighed wet and a 10 % (W/V) homogenate was prepared by homogenising the flukes in ice-cold 0.25 M sucrose solution containing 0.15 M Tris-HCl (pH 7.5) using a tissue homogeniser in an ice-bath. This homogenate was centrifuged at 1000 rpm for 10 min and the sediment containing the cellular particles viz. nucleus and other organelles were discarded. The supernatant was used as the enzyme source.

Preparation of enzyme samples

The cytosolic fractions of *C. cotylophorum* were prepared following the method of Fry *et al.* [16]. The sample prepared as described earlier, was centrifuged at 10,000 rpm for 20 min and the supernatant thus obtained was the cytosol fraction. All the centrifugation steps were carried out at4 °C using refrigerated ultracentrifuge (REMI C 24).

Assay of pyruvate kinase

Pyruvate kinase (PK) (EC 2.7.1.4) activity in the cytosolic fraction was assayed following the method of McManus and Smyth [17]. PK catalyses the inter conversion of phosphoenolpyruvate (PEP) and pyruvate. The reaction mixture contained 1 ml of 300mM Tris-HCl buffer (pH 7.8) [18], 0.5 ml of 42 mM magnesium sulphate (MgSO4), 0.5 ml of 450 mM potassium chloride (KCl), 0.3 ml of 50 mM adenosine diphosphate (ADP), 0.3 ml of 50 mM PEP, 0.3 ml of 2 mM nicotinamide adenine dinucleotide reduced (NADH), 0.025 ml of 48 mM fructose biphosphate (FBP), 0.025 ml of 15 units of lactate dehydrogenase (LDH) and 0.05 ml of enzyme sample. The reaction was started by the addition of the enzyme sample and the decrease in absorbance at 340 nm was recorded for 3 min at an interval of 15 sec. The enzyme activity was calculated from the millimolar coefficient of 6.22 for NADH and was expressed in n moles NADH oxidised /min /mg protein.

Assay of phosphoenolpyruvate carboxykinase

The activity of phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32) was assayed according to the method of McManus and Smyth [17]. PEPCK catalyses the formation of oxaloacetate (OAA) from PEP. The assay mixture contained 1ml of 300mM imidazole buffer (pH 6.2) [18], 0.4 ml of 300 mM MgSO4, 0.3 ml of 400 mM KCl, 0.3 ml of 70 mM sodium bicarbonate ($NaHCO_3$), 0.3 ml of 20 mM ADP, 0.3 ml of 40 mM PEP, 0.3 ml of 2 mM NADH, 0.05 ml of 15 units of malate dehydrogenase (MDH) and 0.05 ml of enzyme sample. The reaction was started by the addition of the enzyme sample and the decrease in absorbance was read at 340 nm for 3 min at an interval of 15 sec. The enzyme activity was calculated from the millimolar coefficient of 6.22 for NADH and was expressed in n moles NADH oxidised/min/mg protein. Protein in the samples was determined by the method of Lowry *et al.* [19]. The data were statistically analyzed using the statistical software SPSS version 16.0.

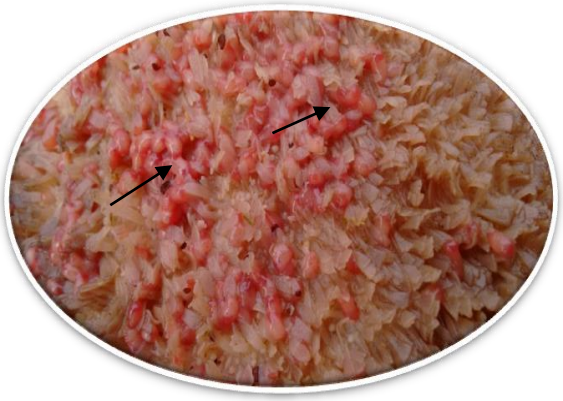

Fig. 1 *Cotylophoron cotylophorum*

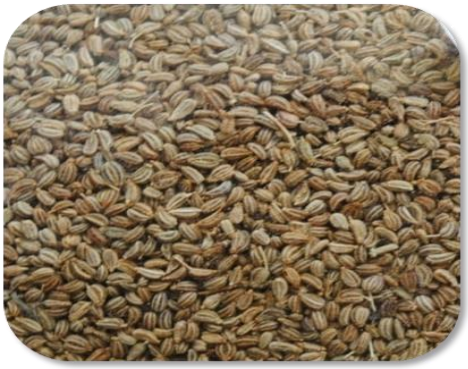

Fig. 2 *Trachyspermum ammi*

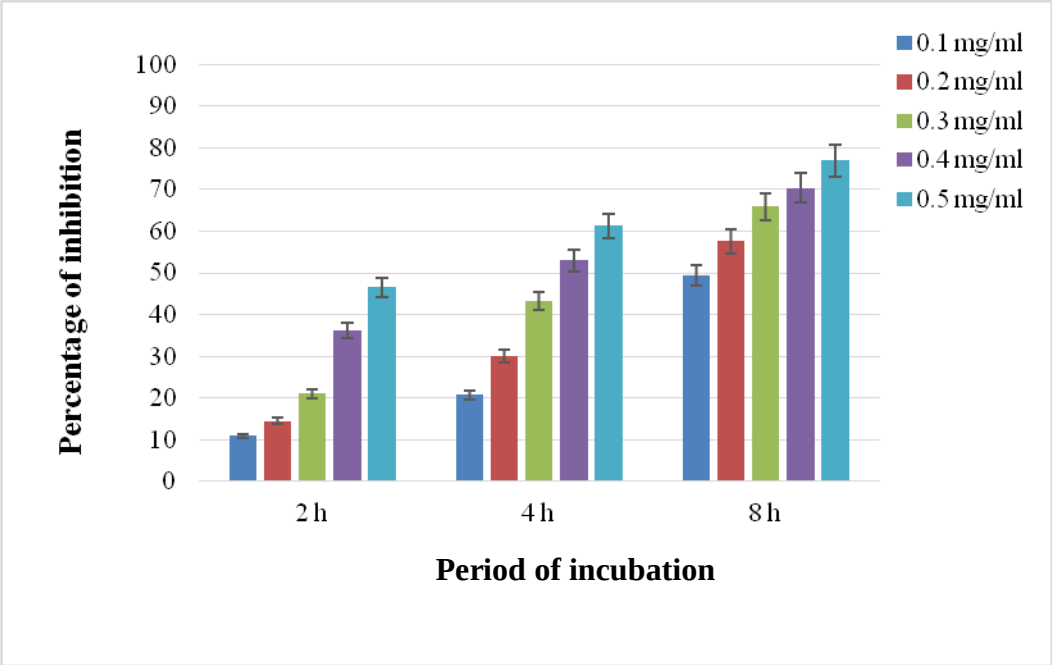

Fig. 3 Effect of *Ta*EE on PK activity of *C. cotylophorum*

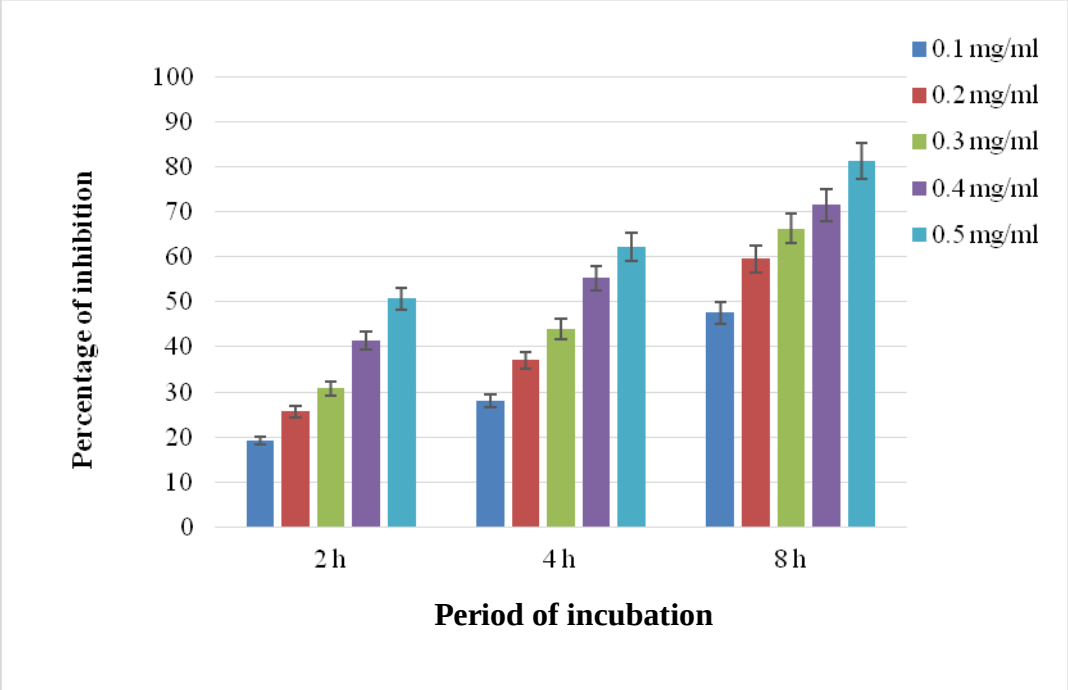

Fig. 4 Effect of *Ta*EE on PEPCK activity of *C. cotylophorum*

One-way Anova using Bonferroni test was applied to find out the significant difference between the different concentrations of plant extract and period of exposure.

RESULTS:

*Ta*EE significantly inhibited both the enzymes PK and PEPCK of *Cotylophoron cotylophorum*. PK activity was found to be highly inhibited by 46.51%, 61.23% and 76.91% at 0.5 mg/ml concentration of *Ta*EE after 2, 4 and 8h respectively (Fig. 3). The maximum level of inhibition in PEPCK was observed in *Ta*EE-treated flukes 50.67%, 62.23% and 81.30% after 2, 4 and 8h of incubation at 0.5 mg/ml concentration (Fig. 4). The present study clearly indicated that the *Ta*EE having inhibitory effect on PK and PEPCK, of *C. cotylophorum*. The percentage of inhibition of both PK and PEPCK was statistically significant ($P<0.005$).

DISCUSSION

Carbohydrate metabolism in parasites plays an important role in its adaptation and survival in mammalian host. The major difference between carbohydrate catabolism of parasites and the host was acetyl-CoA, which plays the same prime role in parasites [20]. The present study revealed that the inhibitory effect of ethanol extract of *Trachyspermum ammi* on PK and PEPCK activity of *Cotylophoron cotylophorum*. Similarly inhibitory effects of various synthetic and plant based anthelmintics on PK and PEPCK of trematodes and nematodes were well documented [21,18,22 - 25]. The enzymes PK and PEPCK are functionally linked. The two enzymes compete for phosphoenolpyruvate (PEP) the common substrate which directs it to aerobic (PK) and anaerobic (PEPCK) pathways [26]. Inhibition of PK results in decreased concentrations of pyruvate and PEP which ultimately result in decline production of ATP. Similarly the inhibition of PEPCK results in decreased concentration of oxaloacetate (OAA)

which reduces the generation of ATP. The synthesis of either lactate or succinate is contained by the competing activities of PK and PEPCK in anaerobic metabolic pathway. The carbon flow from PEP into the final products of anaerobic metabolism was directed by PK and PEPCK [27]. Thus the enzymes compete for the substrate PEP, and their relative activities account for the PEP-lactate or acetate/PEP-succinate or propionate pathways [28]. The succinate formation has a distinct advantage for the parasite over the LDH reaction in the anaerobic habitat of intestinal parasite, as the production of succinate is the end step in mitochondrial of metabolic pathway, which catalyses the transfer of electrons from NADH to fumarate, and fumarate serves as an end product of electron receptor. Inhibition of both PK and PEPCK activities arrests the PEP-lactate and PEP-succinate pathways. Thus the energy yielding process is impaired and deprives the parasite of its ATP production. Decreased production of ATP may lead to the elimination of parasite from the host [29,30]. The present investigation clearly elucidated the anthelmintic efficacy of *T. ammi* seeds against *C. cotylophorum*. Further identification of effective phytochemicals is necessary for the development of potential phytotherapeutic drug formulation for control the ruminal paramphistomes.

REFERENCES:

1. Soulsby, E. J. L., 2009. Helm in the Arthropod and Protozoa of domestic animals. 7. London: *Bailliere Tindall.,* **pp.** 809.
2. Madzingira, O., Mukaratirwa, S., Pandey, V. S. and Dorny, P., 2002. A questionnaire survey of the management and use of anthelmintics in cattle and antelope in mixed farming systems in Zimbabwe. *J. of the South African vet. Ass*. **73**: 70 – 73.
3. Fischoeder, F., 1901. Die paramphistomiden der saiigethiere. *Zool. Anx,* **24**: 365-375.
4. Veerakumari, L.., 1996. *In vitro* studies on the effect of some anthelmintics on *Cotylophoron cotylophorum* (Fischoeder, 1901) (Digenea: Paramphistomidae). A structural and biochemical analysis. Ph.D. thesis, University of Madras. Chennai.
5. Palanivel, K. M., Rishikesavan, R., Sureshkumar, K. and Gopi, H., 2012. Epidemiology of parasitic infection in Madras red sheep in Tamil Nadu. *Int. J. of Food, Agri. Vet. Sci*., **2(3):** 140-145.
6. Hassan, S. S., Kaur, K., Joshi, K. and Juyal, P. D., 2005. Epidemiology of paramphistomosis in domestic ruminants in different district of Punjab and other adjoining areas. *J. Vet. Parasitol*., **19**: 43-46.
7. Tariq, K. and Tantry, M., 2012. Preliminary studies on plants with anthelmintic properties in Kashmir–The North–West temperate Himalayan region of India. *Chinese Med*., **3(2)**: 106–112.
8. Arabshahi – Deloues, S. and Urooj, A., 2007. Antioxidant properties of various solvent extracts of mulberry (Moris indica L.) leaves. *Food chemistry,* **102**: 1233-1240.
9. Tshikalange, T. E., Meyer, J. J. and Hussain A. A., 2005. Antimicrobial activity, toxicity and isolation of a bioactyive compound from plants used to treat sexually transmitted disease. *J. Ethnopharmacol.,* **96 (3)**: 515-519.
10. Kamal Jeet, Nisha Devi, Thakur Narender, Tomar Sunil, Shalta Lalit and Thakur Raneev, 2012. *Trachyspermum ammi* (ajwain): a comprehensive review. *Int. Res. J. Pharm*., **3(5)**. **pp**. 133-138.

11. Srivastava, K. C., 1988. Extract of a spice Omum (*Trachyspermum ammi*) shows antiaggregatory effects and alters arachidonic acid metabolism in human platelets. *Prostaglandins Leukot Essent Fatty Acids.* **33**: 1-6.
12. Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N., and Shaaya, E., 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: Possible mode of action against insect pests, *Pest Management Sci.,* **58**: 1101–1106.
13. Kwon Park, I., Junheon, K. and Sang-Gil, L., 2007. Nematicidal Activity of plant essential oils and components from Ajwan (*Trachyspermum ammi*), all spice (Pimentadioica) and Litsea (Litseacubeba) Essential oils against Pine wood Nematode (*Bursaphelenchus xylophilus*). *J. of Nem.* **39(03)**, 275–279.
14. Sadiq, U., Asif, M., Sajad Mir, Meraj, A. Md., Hussain, U. and Wasim, A., 2012. Anti-inflammatory and antioxidant activity of *Trachyspermum ammi* seeds in collagen induced arthritis in rats. *Int. J. Drug Dev. & Res.,* **4 (1):** 210-219.
15. Pallewad, S., Sanjay Shamrao Nanware and Dhanraj Balbhim Bhure, 2015. Biochemical contents of *Cotylophoron cotylophorum* (Fischoeder, 1901) stiles et Goldberger, 1910 and its host intestinal tissue. *Biolife*., **3(1):** 192-195.
16. Fry, M., Bazil, C. and Jenkins, D. C., 1983. A comparison of mitochondrial electron transport in the intestinal parasitic nematodes *Nippostrongylus brasiliensis* and *Ascardia galli. Comp. Biochem. Physiol.,* **75:** 451-453.
17. McManus, D. P. and Smyth, J. D., 1982. Intermediary carbohydrate metabolism in protoscoleces of *Echinococcus granulosus* (horse and sheep strains) and *E. multiocularis. Parasitol*., **84**: 351-366.
18. Priya, P. and Veerakumari, L., 2011. *In vitro* effect of *Acacia concinna* on the key enzymes of carbohydrate metabolism of *Cotylophoron cotylophorum* (Fischoeder, 1901). *Biomed.,* **31(3)**: 329– 33.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., 1951. Protein Measurement with the Folin Phenol Reagent. *The J. of Biol.Chem.,* **193**: 265-275.
20. Kosalge, S. B, Fursule, R. A. 2009. Investigation of *in vitro* anthelmintic activity of *Thespesia lampas* (Cav.). *Asian J. Pharm. Clin. Res.,* **2(2)**: 69-71.
21. Navaneetha, L. K. and Veerakumari L., 2009. Effect of *Allium sativum* on the phosphoenolpyruvate carboxykinase and pyruvate kinase activity of *Haemonchus contortus in vitro. Pharmacogn Mag.,* 5(20):430–32.
22. Roy, B., Swargiary, A. and Giri, B. R., 2012. *Alpinia nigra* (Family Zingiberaceae): An anthelmintic medicinal plant of North–East India. *Adv. Life Sci*., **2(3)**: 39–51.
23. Swargiary, A., Roy, B., Giri, B. R. and Rohang, B., 2013. A comparative study on the anthelmintic efficacy of some medicinal plants of North–East India: alteration in the glycolytic enzymes of *Fasciola buski*, a giant intestinal fluke. *Asian Pac. J. Trop. Med.*, 412–20.
24. Veerakumari, L., Lalhmingchhuanmawii, K. and Ashwini, R., 2014. Effect of *Punica granatum* ethanol extract

on the carbohydrate metabolism of *Cotylophoron cotylophorum*. *Joseph's Int. J. of Biol. Sci.,* **1(1)**: 1-5.

25. Jeya, S. and Veerakumari. L., 2016. Effect of *Piper betle* on pyruvate kinase and phosphoenolpyruvate carboxykinase of *cotylophoron cotylophorum. WJPPS.* Vol. 5, **(1)**: 1537-1548.
26. Bueding, E. and Saz, H. J., 1968. Pyruvate kinase and phosphoenolpyruvate carboxy-kinase activities of *Ascaris* muscle, *Hymenolepis diminuta* and *Schistosoma mansoni*. *Comp. Biochem. Physiol.,* **24**: 511–18.
27. Burenina, E. A., 2004. *Turbellaria Phagocata sibirica*: Some enzymes of carbohydrate and energy metabolisms. *J. Evol. Biochem. Phys.*, **40(4):** 374–383.
28. Davila, C., Malagon, D., Valero, A., Benitez, R. and Adroher, F. J., 2006. Anisakis simplex: CO2-fixing enzymes and development throughout the *in vitro* cultivation from third larval stage to adult. *Exp. Parasitol.,* **114**: 10-5.
29. Murray. P. R., 2013. Medical Microbiology, Seventh Edition, ISBN: 978–0– 323–08692–9, an imprint of Elsevier Inc.
30. Lokesh, R. and Veerakumari, L., 2016. Effect of ethanol extract of *Acorus calamus* on Pyruvate kinase and Phosphoenolpyruvate carboxykinase activity of *Cotylophoron cotylophorum. Int. J. Pharm. Bio. Sci.,* 2016 April; 7(2): (P) 164-168.