International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Effect of *Adhatoda vasica* and *Piper Betle* Leaves Extracts on Motility and Acetylcholinesterase of *Cotylophoron Cotylophorum Invitro*

Jeya S[1], Veerakumari L[2]

[1]Corresponding Author, Department of Advanced Zoology and Biotechnology, Meenakshi College for Women (Autonomous), Chennai 600 024, India

[2]PG and Research Department of Zoology, Pachaiyappa's College, Chennai 600 030, India

Abstract: ***Parasitism by gastrointestinal trematodes is one of the major constraints on livestock production all over the world. Paramphistomosis is commonly known as stomach fluke disease, caused by infestation of ruminants with paramphistome species. Cotylophoron cotylophorum is one among the paramphistome species, which cause the disease Paramphistomosis. The clinical symptoms of this disease include weight loss, loss of production and anemia; immature flukes cause diarrhoea in sheep and goats. The current scenario of plant based drugs for various ailments of human beings as well livestock instigated us to carry out the present study which is a sincere attempt to reveal the anthelmintic activity of solvent extracts of Adhatoda vasica (Av) and Piper betle (Pb) leaves against C. cotylophorum. Motility and acetylcholinesterase (AChE) are taken as biomarkers to identify the anthelmintic efficacy of Av and Pb. Gross visual observations of motility of the parasites against various solvent extracts revealed that the ethyl acetate extract of both plants (AvEaE, PbEaE) were effective. The flukes were exposed to five different sub lethal concentrations of AvEaE and PbEaE (0.01, 0.05, 0.1, 0.5 and 1.0mg/ml) for 2h, 4h and 8h. Highest percentage of inhibition in motility and AChE was found in drug treated parasites after 8 h of exposure. AChE is an important enzyme in helminths which is associated with the neuromuscular coordination. The inhibition of AChE could be the significant reason for the drastic changes in motility of the parasites. Results of this study clearly showed that the extracts of AvEaE and PbEaE had immolated the parasites by affecting their neurotransmission and dismantle the muscular coordination system.***

Keywords: Paramphistomosis, *Cotylophoron cotylophorum*, *Adhatoda vasica*, *Piper betle*, Acetylcholinesterase

1.Introduction

Helminths are multicellular pathogens which infect considerably high proportion of global human and domestic animal population. Gastrointestinal trematode and nematode infection is one of the major problems in the world especially in tropical and subtropical countries. Paramphistomosis is caused by a number of closely related amphistome species of various genera. Several clinical outbreaks of paramphistomes in domestic ruminants were recorded in different places by various workers. The prevalence of paramphistomosis infection among cattle in Andhra Pradesh was high during rainy season [1]. Among several species of amphistome *C. cotylophorum* is more prevalent in Tamil Nadu [2]. It is a digenetic trematode that parasitizes the rumen and reticulum of livestock. Adults destroy part of rumen and cause inflammation of the intestine [3]. Subclinical infections of gastrointestinal parasites decrease feed intake, body-weight gain, milk and wool production. The use of anthelmintic which is foremost among the control measures against parasites faces serious setback as a result of the emergence and spread of drug-resistant gastrointestinal parasites [4], [5] and [6]. Above all the costs of commercially available anthelmintic drugs are relatively high [7].Farmers from developing country are not willing to spend their meager income to purchase these drugs for regular treatments [8]. This necessitates the search for alternative chemotherapeutic agents that are economical, readily available and to which parasites are unlikely to develop resistance [9].

2.Background of the Study

In today's world, more than half of the human population suffers from various types of infection while majority of cattle suffer from worm infections [10]. Due to the important economic impact of gastrointestinal parasitic nematodes in the livestock industry around the world, most of the researches on plant extracts are being focused on searching bioactive compounds from plants against this important group of parasites. Traditionally, some plants around the world are well known as anti-parasitic plants because they contain substances with anthelmintic effects against parasitic nematodes affecting agricultural crops [11]. A wide range of plants and their products around the world are being explored to look for their possible anthelmintic effects on cestodes and trematodes [12], and against nematodes [13]. *Adhatoda vasica* belonging to Acanthaceae family is important medicinal herb has been commonly used in the indigenous system of medicine of Naga tribes in India for curing parasitic intestinal worm infections. Chemical constituents of *A. vasica* leaves are vasicine, vasicinol, vasicol, vasicinone, adhatonine and pyrroloquinazoline alkaloids, etc., [14]. *Piper betle* an indigenous medicinal plant has a folk (Siddha and Ayurveda) reputation in the rural areas of southern India, is a member of the family Piperaceae. Significance of *P. betle* leaves have been explains in relation to each and every plethora of human life from the dawn of civilization [15]. *P. betle* leaves contain caryophyllene, cadinene, γ-lactone, allyl catechol, *p*-cymene and eugenol methyl ether in varying amounts [16] and [17]. Later study also revealed that the presence of cepharadione

A, dotriacontanoic acid and tritriacontane from the petrol extract of leaves of *Piper betle* [18]. However, the anthelmintic efficacy of these plants against paramphistomes was not assessed. The most often used and reliable parameter to find the anthelmintic property of plant material is the motility of the parasites. Motility is purely based on neuromuscular coordination [19]. AChE is an important enzyme in helminths which is associated with the motility of the parasites and their neuromuscular coordination. The neuromuscular system of the parasites is sensitive to many anthelmintic drugs [20]. In previous investigations efficacy of drugs has been evaluated by gross visual observation on the motility response and level of AChE of drug treated parasites as comparison made with controls [21], [22], [23] and [24].

In this line the present study focused on *in vitro* effect of *Adhatoda vasica* and *Piper betle* leaves on motility response and AChE against *Cotylophoron cotylophorum*, a trematode parasite of ruminants.

3.Materials and Methods

3.1 *In vitro* maintenance of *Cotylophoron cotylophorum*

Hedon-Fleig solution (pH 7.0) is the best medium for *in vitro* maintenance of *C. cotylophorum* [25]. It is prepared by dissolving 7 g of sodium chloride, 0.1 g of calcium chloride, 1.5 g of sodium bicarbonate, 0.5 g of disodium hydrogen phosphate, 0.3 g of potassium chloride, 0.3 g of magnesium sulphate and 1 g of glucose in 1000 ml of distilled water. Adult live flukes were collected from the rumen of the sheep slaughtered at a local abattoir in Chennai. The flukes were washed thoroughly in physiological saline and maintained in Hedon-Fleig solution (pH 7.0).

3.2 Extract preparation of plant material

Leaves of *A. vasica* and *P. betle* were collected from local market and shade dried for 21 days. The dried leaves were coarsely powdered. Extraction was done following the standard method [26]. The coarsely powdered leaves of *A. vasica* and *P. betle* were soaked successively in hexane, chloroform, ethyl acetate and ethanol for 48 h, aqueous extract was also prepared. Intermittent agitation was necessary while soaking in various solvents. Watt man filter paper no1 was used for filtrations. Distillation was done with rotary evaporator (EQUITRON). Extracts were kept in Lyodel lyophilizer (DELVAC) for drying.

3.3 Gross visual observation on the motility of the flukes

Solvent extracts of different concentrations (1, 3 and 5 mg/ml) were prepared with Hedon-Fleig solution (pH 7.0). The motility of the flukes was observed at various time intervals (5, 15, 30 min, 1, 2, 4, 6, 8, 12 and 24 h) of exposure. Simultaneously, control was also maintained in Hedon-Fleig solution without the plant extracts. Based on the motility of the flukes, the observations were categorized as very active (++++), moderately active (+++), slightly active (++), sluggish (+) and dead (-). The flukes with no movement were regarded as dead. Based on the motility of the flukes the effective solvent plant extract was identified, and then five different sub-lethal concentrations were selected (0.01, 0.05, 0.1, 0.5 and 1.0 mg/ml) for further studies.

3.4 Quantitative survey of motility response of drug treated flukes using Electronic Micromotility Meter (EMM)

The flukes were incubated for 2, 4 and 8 h in the various sub-lethal concentrations of the effective solvent plant extracts for further *in vitro* studies. The motility response of the control and drug treated flukes were recorded with the aid of Electronic Micro motility Meter (EMM) [27]. The percentage of inhibition of motility of control and drug treated flukes were calculated by using the formula,

$$\% \text{ inhibition of motility} = \frac{C - T}{C} \times 100$$

Where,
C - Deviation of voltage signal in the control fluke
T - Deviation of voltage signal in the fluke treated with plant extracts

3.5 Quantitative measurement of Acetylcholinesterase:

Acetylcholinesterase (AChE, EC 3.1.1.7) activity was assayed using photometric method [28]. AChE in the sample hydrolyses acetylcholine, which is the substrate and forms thiocholine that will react rapidly and irreversibly with 5-thio-bis-nitrobenzoicacid. The increase in colour intensity was measured spectrophotometrically at 412 nm. The enzyme samples were prepared by homogenizing 500 mg of control and drug-treated flukes in 1 ml of 0.1M phosphate buffer (pH 8.0). The homogenate was centrifuged at 1000 rpm for about 5 min. To 100 µl of the supernatant, 1.3 ml of 0.1 M phosphate buffer (pH 8.0) and 0.05 ml of 0.01M 5-thio-bis-nitro benzoic acid (DTNB) solution was added and transferred to quartz cuvette. The absorbance at 412 nm was set to zero in a UV visible double beam bio spectrophotometer. Later 0.02 ml of 0.075 M acetylthiocholine iodide was added to the reaction mixture in the cuvette and mixed well and the absorbance was noted for 5 min at 15 seconds interval. The increase in absorbance per minute was calculated. AChE activity was calculated using the formula

$$R = 5.74 \times 10^{-4} \times \frac{\Delta A}{\text{Protein content of the sample}}$$

Where,
R - Rate of activity
Δ A- Change in absorbance
The protein content of the sample was estimated following the procedure of Lowry [29]. The enzyme activity was expressed as number of moles of acetylthiocholine iodide hydrolyzed/min/mg protein.

3.6 Statistical analysis

All the data were expressed as mean ± SD. The significance of drug-induced inhibition of the drug-treated flukes was assessed using, analysis of variance (ANOVA) for different concentrations ($P<0.001$) of *Av*EaE and *Pb*EaE. The SPSS software package version 16.0 was used to test the

significance of experiments performed.

4. Results and Discussion

The gross visual observations on motility response of the *Av*EaE and *Pb*EaE treated flukes are presented in tables 1 and 2. Results revealed that the ethyl acetate extracts of *Adhatoda vasica* and *Piper betle* leaves were found more effective. Hence five sub lethal concentrations 0.01, 0.05, 0.1, 0.5, and 1.0 mg/ml of *Av*EaE and *Pb*EaE were selected for further *in vitro* studies. However gross visual observations may have its own pits and fall, for more authentication EMM study was made. The percentage of inhibition on motility response of drug treated flukes recorded with the aid of EMM is shown in tables 3 and 4. The present study elucidated the deleterious effect of *Av*EaE and *Pb*EaE on the motility and AChE of *C. cotylophorum*. The percentage of inhibition on motility by *Av*Ea are 35.05, 69.23 and 92.64% and by *Pb*Ea are 52.38, 65.38 and 95.06%, after 2, 4 and 8h exposure at higher sub- lethal concentration (1.0mg/ml); which indicates that both the plant extracts have higher potency against *C. cotylophoroum.* AChE is an enzyme that catalysis the hydrolysis of the neurotransmitter acetylcholine (ACh) to acetate and choline, and plays a crucial role in terminating the Ach, the neurotransmitter that is essential for nerve transmission at the cholinergic synapse. Previous studies by various investigators stated that the most of the anthelmintic compounds act on neuromuscular system by targeting AChE. Similarly, in this study *Av*Ea and *Pb*Ea leaves extracts significantly inhibited AChE of *C. Cotylophorum* (tables 5 and 6) ($P<0.001$). Most parasites rely on neuromuscular coordination for resisting host's intestinal propulsive forces and for absorption and distribution of food for them [30]. Above all the inhibition of AChE causes hyper excitation and desensitization of the muscle receptor resulting in paralysis [31]. In parasitic helminths roles of AChE in altering cell membrane permeability or acting as anti-coagulants, or to increase nutrient availability, have all been proposed by Lee [32]. In another study exposure of *Schistosoma haematobium* to acetylcholine *in vitro* alters the rate of glucose uptake, an effect that is concentration dependent and can be eliminated by inhibitors of the surface AChE or an acetylcholine receptor [33]. AChE is found closely associated with central and peripheral nervous components in cestodes [34]. The considerable loss of AChE activity was found in the cestodes *Raillientina echinobothrida* after treated with crude root peel extract of *Flemingia vestita* [35]. *Erythrina variegate, Cassia auriculata* and *Dioscorea bulbifera* extracts have shown significant anthelmintic activity by producing paralytic effect much earlier and the time to death was shorter against *Eicinia fetida* and *Raillietina spiralis* [36].

Thus results of present investigation clearly declared that the efficacy of *Av*Ea and *Pb*Ea extracts on motility and AChE of *C.cotylophorum.* In recent investigation on *Schistosoma haematobium* the mode of action of anthelmintics is to block the action of acetylcholinesterase, leading to the excessive buildup of the neuro transmitter, acetylcholine. They block cholinergic nerve transmission by stereo specific binding with a receptor, resulting in muscular paralysis [37]. The significant reduced level of AChE could be the main cause of the drastic change brought in motility of drug treated flukes. Furthermore the concentration of the plant extract and duration of exposure to the drug are playing vital role in inhibition of motility among drug treated flukes. The most common effect of anthelmintic drugs against fluke infections is bring out the paralysis of the musculature by inhibiting the action of enzyme involved in neurotransmission. Hence the inhibition of AChE is a kind of placing obstacle between neurotransmission and muscular coordination by the drug which lead fatal path for the parasites.

5. Conclusion

The gastro-intestinal helminths becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases; hence there is an increasing demand towards natural anthelmintic [38]. The medicinal plants are rich in phytochemicals (secondary metabolites) and essential oils of therapeutic importance. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds [39]. Development of novel efficacious drugs or drug combinations for the treatment of helminthic infections is of high research priority [40]. Hence more research on combinations of different plant based anthelmintics would reveal more avenues in control of helminths infections and ensure the safety of herbal medications in livestock. The present study demonstrated that the ethyl acetate extracts of *A. vasica* and *P. betle* leaves shown promising anthelmintic activities, the difference in their anthelmintic activities against the flukes may be attributed to their difference in their phytoconstituents. From the observations, it can be concluded that the leaves of *Adhatoda vasica* and *Piper betle* are the good sources of natural drugs and might be useful in treating the diseases associated with stomach fluke infections. Further study is needed for isolation, purification and identification of potential compounds from both plant extracts and also *in vivo* studies are mandatory for practical utility of these suggested phytomedicine against *Cotylophoron cotylophorum*.

Table 1: Gross visual observation on the motility of *C. cotylophorum* exposed to various extracts of *A. vasica*

Medium of extract	concentrations mg/ml	5min	15min	30min	1h	2h	4h	6h	8h	12h	24h
Control		++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
*Av*HE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
	3	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
	5	++++	++++	++++	++++	++++	++++	+++	+++	++	+
*Av*CE	1	++++	++++	++++	++++	++++	++++	+++	++	+	-
	3	++++	++++	++++	+++	+++	+++	+++	+	-	-
	5	++++	++++	+++	+++	++	++	++	+	-	-
*Av*EaE	1	++++	++++	++++	++++	+++	++	++	+	-	-

	3	++++	++++	++++	+++	++	++	+	-	-	-
	5	++++	++++	+++	++	++	+	-	-	-	-
	1	++++	++++	++++	++++	++++	+++	+++	+++	+++	++
	3	++++	++++	++++	++++	++++	++++	++++	++++	++	-
*Av*EE	5	++++	++++	++++	+++	+++	++++	+++	+++	+	-
	1	++++	++++	++++	++++	++++	+++	++++	++++	+++	++
	3	++++	++++	++++	++++	+++	+++	++++	+++	++	-
*Av*AqE	5	++++	++++	++++	+++	+++	+++	+++	++	+	-

very active (++++), moderately active (+++), slightly active (++), sluggish (+) and dead (–).

*Av*HE - *Adhatoda vasica* Hexane extract; *Av*CE - *Adhatoda vasica* Chloroform extract; *Av*EaE - *Adhatoda vasica* Ethyl acetate extract; *Av*EE - *Adhatoda vasica* Ethanol extract; *Av*AqE ; *Adhatoda vasica* Aqueous extract.

Table.2 Gross visual observation on the motility of *C. cotylophorum* exposed to various extracts of *P. betle*

Extract	Concentrations mg/ml	5min	15min	30min	1h	2h	4h	6h	8h	12h	24h
Control		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	-
	3	++++	++++	++++	++++	++++	++++	+++	+++	+	-
*Pb*HE	5	++++	++++	++++	++++	++++	+++	++	++	-	-
	1	++++	++++	++++	++++	++++	++++	++++	+++	-	-
	3	++++	++++	++++	++++	+++	+++	+++	++	-	-
*Pb*CE	5	++++	++++	+++	++	++	++	++	+	-	-
	1	++++	++++	++++	+++	+++	+++	++	+	-	-
	3	++++	++++	+++	++	++	++	+	-	-	-
*Pb*EaE	5	++++	++++	+++	++	++	+	-	-	-	-
	1	++++	++++	++++	++++	++++	++++	++++	++++	+++	+
	3	++++	++++	++++	++++	++++	+++	++	+	-	-
*Pb*EE	5	++++	++++	+++	++	++	++	++	-	-	-
	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
	3	++++	++++	++++	++++	++++	++++	++++	++++	+++	++
*Pb*AqE	5	++++	++++	++++	++++	++++	++++	++++	+++	++	+

very active (++++), moderately active (+++), slightly active (++), sluggish (+) and dead (–) .
*Pb*HE - *Piper betle* Hexane extract, *P*bCE - *Piper betle* Chloroform extract, *P*bEaE - *Piper betle* Ethyl acetate extract, *Pb*EE - *Piper betle* Ethanol extract, *P*bAqE - *Piper betle* aqueous extract.

Table 3: Quantitative estimation of motility of *C. cotylophorum* treated with *Av*EaE using Electronic Micro motility Meter

Concentrations mg/ml	**Period of Incubation**		
	2h	4h	8h
0.01	10.70 ± 0.03	24.72 ±0.04	54.54 ± 0.19
0.05	12.64 ± 0.08	34.76 ± 0.08	67.74 ± 0.04
0.1	18.52 ± 0.08	40.61 ± 0.04	74.19 ± 0.08
0.5	25.40 ± 0.08	55.38 ± 0.04	85.80 ± 0.05
1.0	35.05 ± 0.08	69.23 ± 0.08	92.64 ± 0.04
*** - (mean ± S.D,n=5)**			

Table.4 Quantitative assay of motility of *C. cotylophorum* treated with *Pb*EaE using Electronic Micro motility Meter

Concentrations mg/ml	Period Of Incubation		
	2h	4h	8h
0.01	12.47 ± 0.15	27.14 ±0.01	50.00 ± 0.04
0.05	23.80 ± 0.15	38.33 ± 0.05	66.66 ± 0.01
0.1	36.66 ± 0.13	43.84 ± 0.05	74.42 ± 0.03
0.5	45.00 ± 0.15	57.69± 0.08	82.00 ± 0.03
1.0	52.38. ± 0.15	65.38 ± 0.03	95.06 ± 0.01
* - (mean ± S.D,n=5)			

Table 5: *In vitro* effect of *Av*EaE on AChE (% of inhibition) of *C. cotylophorum*

Concentrations mg/ml	*Period Of Incubation*		
	2h	4h	8h
0.01	20.83 ± 0.02	41.02 ± 0.08	56.77 ± 0.04
0.05	29.16 ± 0.04	59.10 ± 0.03	64.09 ± 0.02
0.1	35.41 ± 0.01	68.92 ± 0.05	77.41 ± 0.01
0.5	43.75 ±0.05	75.48 ± 0.04	84.12 ± 0.03
1.0	47.91 ± 0.02	84.87 ± 0.01	96.19 ± 0.03
*** - (mean ± S.D,n=5)**			

Table 6: *In vitro* effect of *Pb*EaE on AChE (% of inhibition) of *C. cotylophorum*

Concentrations mg/ml	*Period Of Incubation*		
	2h	*4h*	*8h*
0.01	18.55 ± 0.01	52.04 ± 0.03	67.22 ± 0.08
0.05	23.39± 0.02	59.27 ± 0.04	71.00 ± 0.01
0.1	37.00 ± 0.02	65.02 ± 0.08	78.13 ± 0.01
0.5	40.66 ±0.04	75.05 ± 0.09	83.26 ± 0.02
1.0	49.85 ± 0.02	79.70 ± 0.50	98.43 ± 0.05
*** - (mean ± S.D,n=5)**			

References

[1] ShanilaKumari, P. and Hafeez (2005) - Prevalence of Paramphistomosis in cattle in Chittoor district of Andhrpradesh, India- *Journal of Parasitic Diseases*. Vol. 29(1) pp01-08.

[2] Veerakumari, L. (1996). In vitro studies on the effect of some anthelmintics on *Cotylophoroncotylophorum* (Fischoeder, 1901) (Digenea: Paramphistomidae). A structural and biochemical analysis. Ph.D. thesis, University of Madras. Chennai

[3] Radwan, N.A., Khalil, A.I. and Wahdan, A.E, (2012). *In vitro* evaluation of anthelmintic activity of *Allium sativum* against adult *Cotylophoron cotylophorum* (Paramphistomidae), Personal noncommercial use only, *PUJ*, 5(2): 135- 146.

[4] Prichard, R., 1994.Anthelmintic resistance. *Vet.Parasitol*., 54:259-268.

[5] Mwamachi, D.M., J.O. Audho, W. Thorpe and R.L. Baker, 1995. Evidence for multiple anthelmintic resistances in sheep and goats reared under the same management in coastal Kenya. *Vet.Parasitol.*, 60: 303-313.

[6] Waller, P.J., 1997. Anthelmintic resistance. *Vet.Parasitol*., 72:391-405.

[7] Hammond, J.A., D. Fielding and S.C. Bishop, 1997. Prospects for Plant anthelmintics in tropical veterinary medicine.*Vet. Res.Comm*.,21 : 213-228

[8] Kusiluka, L. and D. Kambarage, 1996. Common Diseases of sheep and Goats in Sub- Saharan Africa: Diseases of small Ruminants. A Handbook. 1st Edn, Center for Tropical Veterinary Medicine, Scotland.

[9] Coles, G.C. and R.T. Roush, 1992. Slowing the spread of anthelmintic resistant nematodes of sheep and goats in the United Kingdom. *Vet. Res.*, 130: 505-510.

[10] Chaturvedi, M., Dwivedi, S., Dwivedi, A.,Barpete, P.K. and Sachan, R, 2009. Formulation and evaluation of polyherbal anthelmintic preparation. *Ethnobotanical Leaflets*, 13: 329 – 331.

[11] Krueger, R., Dover, E.K., McSorley, R., Wang, K-H. (2009) Marigold (*Tagetesspp*) for nematode management. University of Florida IFAS Extension, *Publication ENY*-056.

[12] Abdel-Ghaffar, F., Semmler, M., Al-Rasheid, K. A., Strassen, B., Fischer, K., Aksu, G., and Mehlhorn, H. (2011). The effects of different plant extracts on intestinal cestodes and on trematodes. *Parasitology research*, 108(4), 979-984.

[13] Datsu Kalip, R., SlyrandaBaltini, A., Wycliff, A., Abdul rahaman, F.I. (2011) Preliminary phytochemical screening and in vitro anthelmintic effects of aqueous extracts of *Salvadorapersica* and *Terminaliaavicennoides* again ststrongylinenema-todes of small ruminants in Nigeria. *Journal of Animal and VeterinaryAdvances*. 10(4):437-442

[14] Singh, T. P., & Singh, O. M. (2011). Phytochemical and pharmacological profile of *Zanthoxylumarmatum* DC-An overview. *Indian Journal of natural products and resources*, 2(3), 275-285.

[15] Lakshmi, B. S., & Naidu, K. C. (2010). Comparative Morphoanatomy of *Piper betle* L. cultivars in India. *Annals of Biological Research*, 1(2), 128-134.

[16] Baslas, R.K.; Baslas, K.K. Chemistry of Indian essential oils - Part VIII. Flavour Ind. (1970), 1473-474; [Chem. Abstr. 1971, 74, 34538z].

[17] Rastogi, R.P.; Mehrotra, B.N.(1993)*Compendium of Indian Medicinal Plants*, Vol. 3; Publications and Information Directorate: New Delhi,; pp. 502-503

[18] Parmer, V S., Jain,S.C., Gupta,S., Talwar,S., Rajawanshi, V.K., Kumar, R., et al. (1998) Polyphenols and alkaloids from *Piperspecies*. *Phytochemistry*, 49, 1069-1078.

[19] Kumar,D., Chandra, S and Tripathi, H.C., 1995. In vitro motility recording of *Fasciolagigantica*. *J.Vet.Parasitology*., 9:31-36.

[20] Barragry, T. (1984). Anthelmintics – A review: Part II. *N.Z.Vet.J*., 32:191-199.

[21] Jenkins, D. C. & T. S. Carrington. 1982. An *in vitro* screen for anthelmintics employing worms *Nippostrongylusbrasiliensis* in a defined medium. *Veterinary Parasitology* 11: 223-230.

[22] Kumar, D. and Tripathi, H.C. (1998). Paralytic effect of fasciolicidal agents on rhythmic motility of *Fasciolagigantica*. *Indian Journal of Animal Sciences*, 68:1126-1129.

[23] Veerakumari L and Priya P (2006). In vitro effect ofAzadirachtin on the motility and Acetylcholinesterase activity of *Cotylophoron cotylophorum* (Fischoeder, 1901).*Journal of Veterinary Parasitological*, 20: 1-5.

[24] ManojDhanraj and Veerakumari. (2014). In vitro effect of *syzygiumaromaticum* on the motility and Acetylcholinesterase of *Cotylophoron cotylophorum.Ind. J. Vet. & Anim. Sci. Res*., 43 (3) 187 - 194,, May - June 2014

[25] L.Veerakumari, and Munuswamy, N., 1999. In vitro studies on the effects of some anthelmintics on Cotylophoron cotylophorum (Digenea, Paramphistomidae): a structural analysis. Cytobios 98: 39-57.

[26] Harborne JB, (1998). *Phytochemical methods*. In a guide to modern techniques of plant analysis 3rd ed., pp: 40-137

[27] Veerakumari, L. (2003). Micromotility meter: an instrument to evaluate the efficacy of anthelmintic drugs. In: "*Proceedings of Fourteenth National Congress of Indian Association for the Advancement of Verterinary Parasitology"* 1998 Oct. (Maske. D. K., Kolte, S. W., Banubakode, S.B. and Kurkure, N.V.eds.), Nagpur, India, pp.152.

[28] Ellman, G.L., Courtney, K.D., Andrews, V. and Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharamcology*, 1:88-95.

[29] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurements with folin phenol reagent. *Journal of Biology and Chemistry*, 193:265-275.

[30] Maule, A.G., Mousley, A., Marks, N.J., Day, T.A., Thompson, D.P., Geary, T.G. and Halton, D.W. (2002). Neuropeptide Signaling Systems - Potential Drug Targets for Parasite and Pest Control. *Current Topics in Medicinal Chemistry*, 2(7): 733-758.

[31] Opperman, C. and Chang, S. (1992). Nematode acetylcholinesterase: Molecular forms and their potential role in nematode behavior. *Parasitology Today*, 8:406-410.

[32] Lee, D.L. (1996). Why do some nematode parasites of the alimentary tract secret acetylcholinesterase? *InternationalJournal of Parasitology*, 26:499-508.

[33] Camacho, M., and Agnew, A. (1995). Schistosoma: rate of glucose import is altered by acetylcholine interaction

with tegumental acetylcholine receptors and acetylcholinesterase. *Exp. Parasitol*. 81, 584–591. doi: 10.1006/expr.1995.1152.

[34] Maule A G, Halton D W, Shaw C and Johnston, C F.(1993) The cholinergic, serotoninergic and peptidergic components of the nervous system of *Monieziaexpensa*(Cestoda, *Cyclophyllidae*); *Parasitology* 106 429-440

[35] Pal, P., and Tandon, V. (1998). Anthelmintic efficacy of Flemingia vestita (Fabaceae): genistein-induced alterations in the esterase activity in the cestode, *Raillietina echinobothrida*. *Journal ofbiosciences*, 23(1), 25-31.Chicago

[36] Satish B. Kosalge et al. (2009) Investigation of anthelmintic potential of some plants claimed by tribals of satpuda hills. Int.J. PharmTech Res.,1(1)

[37] G. Hrckova and S. Velebny, (2013) Pharmacological Potential of Selected Natural Compounds in the Control of Parasitic Diseases, *Springer Briefs in Pharmaceutical Science & Drug Development*, DOI: 10.1007/9 78-3-7091-1325-7_2.

[38] Sondhi, S.M., Shahu, R., MaganArchana, (1994), *Indian Drugs*, 31(7), 317-320.

[39] Ponnulakshmi, R. and Ezhilarasi, B.S, (2013). Efficacy of bulb extracts of *Allium cepa* varieties (red, white and small onion): An in vitro antifungal and antioxidant activity, *Int. J. Pharm. Bio. Sci*., 4(4): 692 – 713.

[40] Keiser, J., Tritten, L., Adelfio, R., & Vargas, M. (2012). Effect of combinations of marketed human anthelmintic drugs against *Trichuris muris in vitro* and *in vivo*. *Parasites & Vectors*, *5*, 292. doi:10.1186/1756-3305-5-292.

Author Profile

Mrs. Jeya.S is a life member of The Indian Science Congress Association and working as Assistant Professor in the Department of Adv. Zoology and Biotechnology in Meenakshi College for women (Autonomous) Chennai 24. She has fifteen years of experience in teaching; currently she is doing Ph.D. (Part-time) in the field of veterinary Parasitology under the able guidance of Dr.L.Veerakumari.

Dr. (Mrs)L. Veerakumari, Associate Professor & Head, PG & Research Department of Zoology, Pachaiyappa's College, Chennai, Tamil Nadu ,India has thirty five years of teaching and research experience. She has received her PhD degree from University of Madras, India in 1997. She is a life member of Indian Association for the Advancement of Veterinary Parasitology, Indian society of Parasitology, Indian Association of Biomedical Scientists, Indian Association of physiologists and pharmacologists, Indian Society of education and environment, Indian Association of Science and technology and Indian Science Congress Association. She has authored two books and has published many research papers. She has completed six research projects funded by UGC, DST and TNSCST. She is a Gold medalist and received many best paper awards, MABMS and FABMS Title, Best researcher award , Bharat Jothi award, Ismail oration award and Inducted into the American order of Scientific and technical merit.