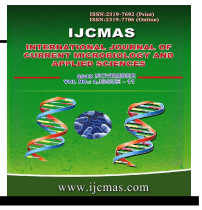
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 11 (2013) pp. 247-255**
http://www.ijcmas.com



Original Research Article

Evaluation of Anthelmintic Activity of Different Leaf and Stem extract of *Sida cordata* burm.f.

A.R. Gulnaz[1] and G. Savitha[2*]

[1]Department of Biochemistry, PRIST University Vallam, Thanjavur-613413, Tamilnadu, India
[2]Department of Chemistry, Maharani's Science College for Women, J.L.B. Road, Mysore, Karnataka, India
Corresponding author:

A B S T R A C T

In the present study different extracts of leaves and stem of *Sida cordata* were evaluated for its anthelmintic activity against *Pheretima posthuma* (Indian earth worm) and compared with standard Albendazole. Various concentrations of the leaves and stem extracts (5, 25, 50,100 mg/ml) respectively were screened for their anthelmintic activity which involved the determination of time of paralysis and time of death of the test worms. It was found out the petroleum ether extract(100mg/ml) showed 62.33 and 78 minutes, water extract (100mg/ml) showed 72.33 and 59.33 minutes, ethanol(100mg/ml) showed 79.66 and 90 minutes and chloroform with 83and 78 minutes for paralysis and death respectively comparable to the Albendazole at the given experimental concentrations. The aqueous extract of stem exhibited a significant anthelmintic activity effect over other extracts at the given experimental concentrations. Dose dependent activity was observed in both leaf and stem extracts but leaves extract showed more significant activity than stem extracts. The present study indicates the potential anthelmintic activity against *Pheretima posthuma* in the leaves extract of the plant. Further need involves the isolation of phytochemical from the leaves responsible for the activity.

Keywords

Sida cordata burm.f; Anthelmintic activity; Albendazole; Solvent extracts; *Pheretima posthuma.*

Introduction

Plant products have been a source of medicinal agents since the ancient time. From the dawn of civilization, people have been using the important biological properties of various plants for treating different diseases. The widespread use of herbal remedies and healthcare preparations, have been described in ancient texts such as the Vedas and the Bible (Hoareau and Da Silva, 1999). During the past few decades, the indigenous or traditional system has gained much importance in the field of medicine. In most of the developing countries, a large number of populations directly or indirectly depend on the

traditional medicines. Although modern medicines are available, herbal medicine retained their image for historical and cultural reasons**.** Traditional medicine is one of the oldest method of curing diseases and infections, by using various plants. A huge percentage of world's population partially or entirely still depends on botanicals to treat human diseases and infections (Caceres *et al.*, 1991). Whole plant and different parts of plant are used to treat various forms of diseases and infections. Plant extracts have been used in folk medical practices for the treatment of various ailments since antiquity (Koppula *et al.,* 2010).

According to World Health about two billion people suffer from parasitic worm infection (Kumar *et al*., 2010). Helminthiasis is still one among the most important human and animal diseases (Lateef *et al*., 2003). During the past few decades, despite numerous advances made in understanding the mode of transmission and treatment of these parasites, there are still no efficient products to control certain helminthiasis. As an important component of complementary and alternative medicine, traditional medicinal plants may be useful to discovery and development of new chemical substance for helminthes control which are generally considered to be very important sources of bioactive substances (Deore *et al*., 2010). Anthelmintic that are obtained from the natural resources may play an important role in the treatment of worm infection with less side effects (Aswar *et al*., 2008).
In the Indian medicine system the medicinal plant *Sida cordata* is in usage to cure various ailments, both the whole plant and various parts of this plant are used. Pedhi marandu is an herbal formulation used by herbal vendors of south India to treat dysentery, diarrhoea, cholera, given orally for only one day (100g/day), in which *Sida cordata* is one of the components (Chitra vaidiu *et al.,* 2009). The whole plant material of *Sida cordata* is used in treatment of chronic liver disorders, 10-20 ml decoction of the whole plant is given daily for one week to relive from joint pain. The root paste along with cow's butter is applied locally to cure piles and just root paste is applied on boils 'khateera' to take out pus (Panthi.M.P *et al.,* 2009) *Sida cordata* (Burm.f.) Borssum (family: Malvaceae) is a small weed found throughout India, usually on the road sides and other waste places. A procumbent, diffuse, much branched hairy herb with a very short main stem and long slender trailing branches that occasionally root at places of contact with the soil, leaves long-petioled, cordate to roundish with stellate hairs; flowers yellow, solitary or in pairs in the axils; fruits schizocarp located within the persistent calyx.

Our earlier phytochemical studies carried out on different solvent extracts of leaf and stem of *Sida cordata* has revealed the presence of primary metabolites like carbohydrates, amino acids, proteins etc and secondary metabolites like the alkaloids, flavonoids, tannin saponins, phenolics, terpeniods, glycosides, emodins, catechins, coumarins anthraquinones etc. (Gulnaz.A.R & Savitha.G. 2013). Our earlier studies have revealed the efficacy of different extracts of leaves and stem of *Sida cordata* to be a potent anti microbial agent, inhibiting the growth of bacterial strains (Gulnaz.A.R & Savitha.G. 2013).Several reports are present on adaptogenic, hepato protective, and rejuvenation and anti ageing properties of *Sida cordata* . This promoted us to investigate the anthelmintic activity of different extracts of leaves and stem of *Sida cordata*, Present study was carried

out to find the anthelmintic activity of different extracts of leaves and stem of *Sida cordata,* against the *Pheretima posthuma* (Indian earth worm) and compared with standard Albendazole.

Materials and Methods

Plant Collection

Fresh plant material leaves & stem of *Sida cordata* was collected from its natural habitat, from the forest region of Somawarpet in Madekeri district Karnataka. The plant was identified and authenticated at National Ayurveda Dietetics Research Institute Bangalore, (voucher no: RRCBI-11748). The collected fresh plant materials (leaves & stem) were washed in water, shade dried at room temperature and then homogenized to fine powder of 40 mesh sizes and stored in airtight bottles at 4°C.

Extraction of Plant Material

About 100gm of each leaf & stem powder were subjected to extraction by a hot percolation method with 150ml of solvents in their increasing polarity (petroleum ether, chloroform, ethyl acetate, ethanol and water respectively), in Soxhlet apparatus. Each solvent extraction step was carried out for 24 hrs. After extraction, the extracts were concentrated by evaporation and stored at 4°C for further study. The dried extracts (residue) were suspended in normal saline containing Tween 20 (1%) and used for screening the anthelmintic activity.

Worm Collection

Pheretima posthuma (Indian earthworm) were collected from vermiculture tank, Maharani's Science College for Women, Mysore, and Karnataka, India. The worms are washed with normal saline & water to remove all faecal matter and were identified by the HOD Department of Zoology, Maharani's Science College for Women, Mysore, and Karnataka, India.

Standard Drug

Albendazole was taken as Standard drug in the present study.

Anthelmintic Activity

The adult Indian earthworms *Pheretima posthuma* has anatomical and physiological resemblance with the intestinal parasites of human beings (Suresh *et al.,* 2011) hence the anthelmintic activity was evaluated on adult Indian earthworms as per Ghosh *et al.,* method (Ghosh.T *et al.,* 2005).For evaluation of anthelmintic activity test samples of the extract was prepared at the concentration of 5, 25, 50,100 mg/ml in Tween 20 (1%) solution diluted with normal saline. Eight groups of nearly equal sized *Pheretima posthuma* (consisting of three earth worms each in triplicate) were released in to 30 ml of experimental formulation, first group serves as normal control which is treated only with normal saline, second group is treated with tween-20 along with normal saline serves as negative control, group three receive standard drug Albendazole at a concentration of 5mg/ml and it serves as standard. Groups 4-8 are treated with extracts of Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Water in different concentration (5mg/ml, 25 mg/ml, 50 mg/ml, and 100mg/ml). All the test solutions and standard solutions were prepared freshly before starting the experiment. The mean time for paralysis was noted when no movement of any sort

could be observed, except when the worm was shaken vigorously, the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli by putting motionless worms in 50^0 C warm water, no movement of worms confirms death. (Dey *et al*., 2012). Death was concluded when the worms lost their motility followed with white secretion and fading away of their body colours (Karale *et al*., 2010). The Test results were compared with reference compound Albendazole treated samples.

Statistical analysis

The result were express as Mean ± SEM.Statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests.

Result and Discussion

The anthelmintic activity of different leaf and stem extract of *Sida cordata* burm.f are shown in table(1-2) and in the figures (1-4).

All the extract of *Sida cordata* leaf and stem exhibited anthelmintic activity in dose dependent manner. Significant activity was seen in the petroleum ether extract at 100mg/ml with 62.33 and 78 minutes for paralysis and death respectively followed by water extract with 72.33 and 59.33 minutes for paralysis and death respectively in leaves. In stem extracts significant anthelmintic activity was observed only in water extract with 75.30 and 62.33 minutes for paralysis and death respectively followed by ethanol with 89.40 and 90.33minutes for paralysis and death respectively extract at the concentration of 100mg/ml. Albendazole did the same at 95.2 and114.8 min, but having a concentration of 5mg/ml.The worms in the control group were remain alive up to 24 hours of observation.

However, in leaf extracts when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations at 5, 25, 50, and 100mg/ml. The petroleum ether extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of water, ethanol, ethyl acetate, chloroform, extracts. Similar observations were made in the anthelmintic activity as well. The effect of extracts on the paralysis (or) helminthiasis of the worm, according to the results may be indicated as petroleum ether > water >ethanol >ethyl acetate >chloroform extracts. In particular the petroleum ether extract of leaf exhibited an increased paralytic as well as helminthiatic effect over albendazole at the given experimental concentrations.

In stem extracts petroleum ether extracts showed least activity, The effect of extracts on the paralysis (or) helminthiasis of the worm, according to the results may be indicated as water> ethanol>ethyl acetate >chloroform>petroleum ether extracts. In particular the water extract of stem exhibited an increased paralytic as well as helminthiatic effect over albendazole at the given experimental concentrations.

Tannins, the secondary metabolite, found in several plants have been reported to show anthelmintic property by several

Table.1 Anthelmintic activity of the extracts of Leaf of *Sida cordata*

S.No	Group	Extract	Dose(mg/ml)	Response	
				Time taken for paralysis (min)	Time taken for death (min)
1	I	Normal control	-------	----	----------
2	II	Negative control	----------	----------	------
3	III	Standard (Albendazole)	5	95.20±0.02	134 .8 ± 0.07
4	IV a IV b IV c IV d	Petroleum ether	5 25 50 100	110.00 ± 0.01*** 94.50 ± 0.50 72.57 ± 0.02*** 62.33 ± 0.70	168.30 ± 0.38 142.00 ± 0.80 110.00 ±0.50* 78.00 ± 0.09*
5	V a V b V c V d	Chloroform	5 25 50 100	158.00 ± 0.20 105.38 ± 0.66 98.30 ± 0.0 83.00 ± 0.20**	198 .0 0 ± 0.42 153 .50 ± 0.07 102 .43 ± 0.42 98.00 ± 0.13**
6 6	VI a VI b VI c VI d	Ethyl acetate	5 25 50 100	160.50 ± 0.38 128.30 ± 0.70 108 .00 ± 0.52 85.50. ±0.00****	186.5 0 ± 0.08 143.00 ± 0.90 98. 45 ± 0. 88** 92.00 ± 0.01***
7	VII a VII b VII c VII d	Ethanol	5 25 50 100	140.00 ± 0.70 115.38 ± 0.50 95.38 ± 0.00** 79.56 ± 0.00***	160.00 ± 0.88 146.50 ± 0.24 116.54 ± 0.58** 90.00 ± 0.01***
8	VIII a VIII b VIII c VIII d	Water	5 25 50 100	128.40 ± 0.52 104.0 9 ± 0.46 86.2 0 ± 0.00*** 72.33 ± 0.02***	148.02 ±0.90 96.00 ±0.46 65.20 ±0.01*** 59 .33 ±0.03***

*Values are Mean ± SEM (n=3 in each group, in triplicates). *p < 0.05, **p< 0.01, *** p< 0.001. One way ANOVA, followed by Dunnett's multiple comparison tests.

Table.2 Anthelmintic activity of the extracts of Stem of *Sida cordata*

S.No	Group	Extract	Dose(mg/ml)	Response	
				Time taken for paralysis (min)	Time taken for death (min)
1	I	Normal control	----------	----------	----------
2	II	Negative control	----------	----------	----------
3	III	Standard (Albendazole)	5	95.20±0.02	114 .8 ± 0.07
4	IV a IV b IV c IV d	Petroleum ether	5 25 50 100	No response No response No response 202.33 ± 0.70	--------- --------- --------- 178.00 ± 0.09
5	V a V b V c V d	Chloroform	5 25 50 100	No response No response 248.30 ± 0.0 183.00 ± 0.20**	--------- --------- ---------- 230.00 ± 0.13
6	VI a VI b VI c VI d	Ethyl acetate	5 25 50 100	No response No response 150.50 ± 0.38 140.30 ± 0.70	------- ------ 196.0 ± 0. 88 170.00 ± 0.70
7	VII a VII b VII c VII d	Ethanol	5 25 50 100	190.00 ± 0.70 156.38 ± 0.00 115.38 ± 0.00 89.40 ± 0.06*	210.00 ± 0.80 169.50 ± 0.50 156.4 0 ± 0.58 98.01 ± 0.10***
8	VIII a VIII b VIII c VIII d	Water	5 25 50 100	150.60 ± 0.92 98.0 9 ± 0.06*** 78.2 0 ± 0.01*** 75.03 ± 0.02**	170.02 ±0.80 156.00 ±0.66 65.20 ±0.00*** 62 .33 ±0.10***

*Values are Mean ± SEM (n=3 in each group, in triplicates). *p < 0.05, **p< 0.01, *** p< 0.001. One way ANOVA, followed by Dunnett's multiple comparison tests.

Figure. 1 Antihelmintic activity of different leaf extracts of S.cordata

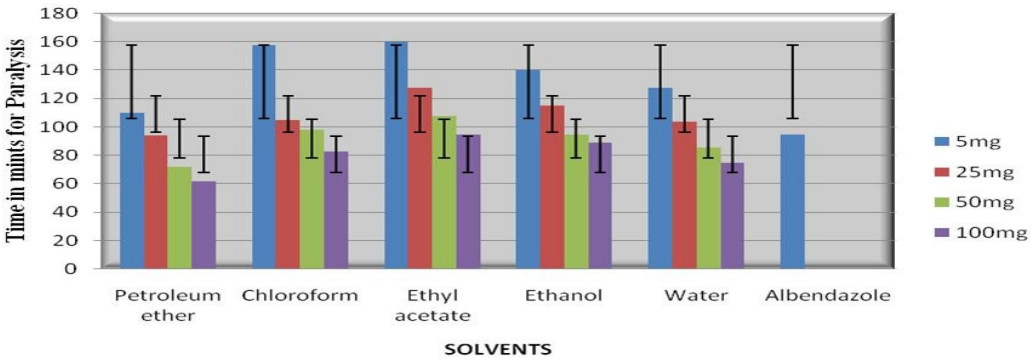


Figure. 2 Antihelmintic activity of different leaf extracts of S.cordata

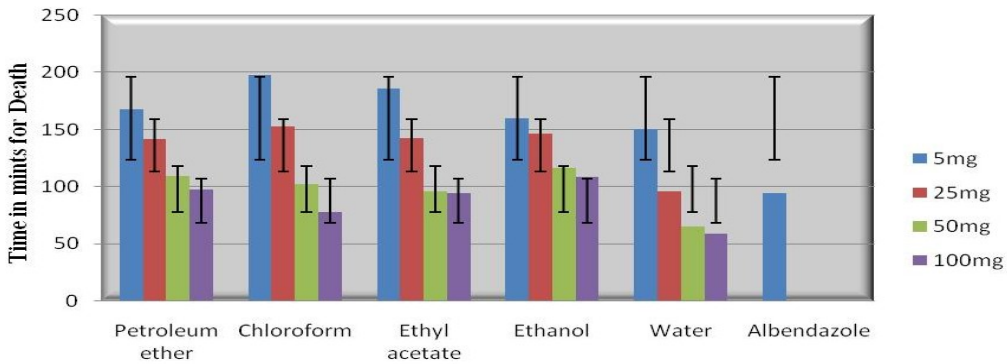
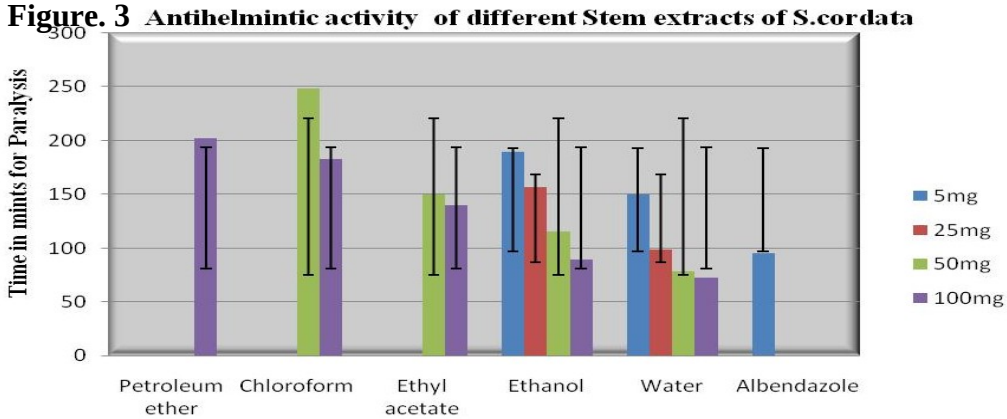
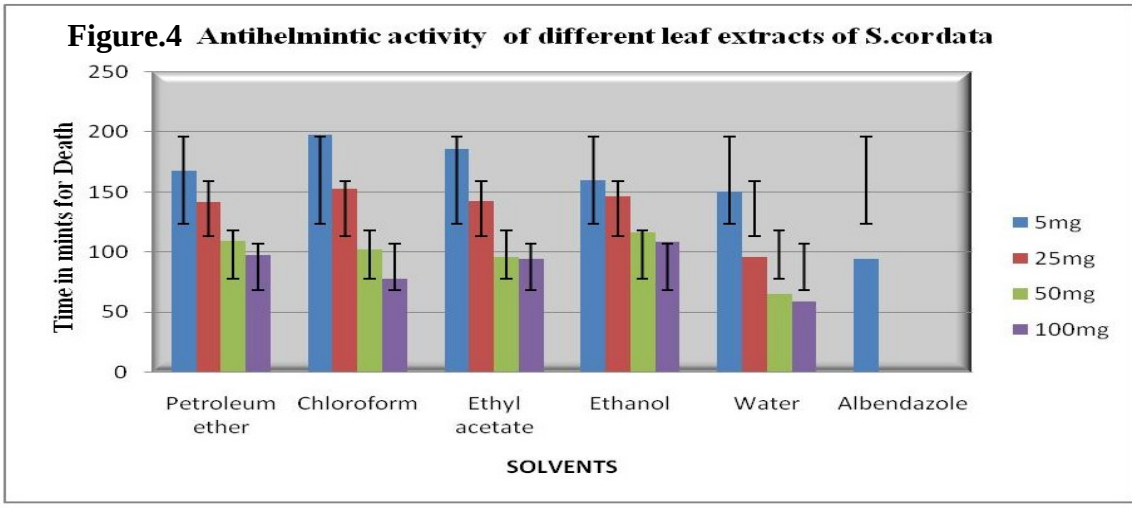


Figure. 3 Antihelmintic activity of different Stem extracts of S.cordata





investigators (Athnasiadou *et al*., 2001, Waller *et al*., 1997). Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or binds to the glycoprotein on the cuticle of parasite or they may interfere with free proteins in the gastrointestinal tract of host animal (Thompson and Geary, 1995) and cause death. The nematode surface is rich in collagen extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is essential for viability (Page and Winter 2003). The mammalian skin also consists largely of collagen in the form of fibrous bundles. Tannins by their reactivity with the collagen matrix results in the loss of flexibility, brings toughness in the skin and hence worms become immobile and non-functional leading to paralysis followed by death. The different extracts of leaf and stem of *Sida cordata* exhibited anthelmintic activity in dose dependent manner. Our earlier phytochemical studies carried out on different solvent extracts of leaf and stem of *Sida cordata* has revealed the presence of primary metabolites like carbohydrates, amino acids, proteins etc and secondary metabolites like the alkaloids, flavonoids, tannin saponins, phenolics, terpeniods, glycosides, emodins, catechins, coumarins anthraquinones etc. (Gulnaz.A.R & Savitha.G. 2013).Possible mechanism for anthelmintic activity may be due to the presence of the secondary metabolites that may bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. The other metabolites may also have a direct effect on the viability on the pre-parasitic stages of helminthes.

Hence further investigation and isolation of the active principles might help in the findings of new herbal drug, which will be effective against various parasitic infections and to establish the mode of action for it.

References

Aswar, M., Aswar U., Watkar B., Vyas M., Wagh A. and Gujar KN, 2008. "Anthelmintic activity of *Ficus benghalensis*" *Inter. J.Green Pharm.*

Athnasiadau, S., I.kyriazakis, F.jackson, R.L.coop. 2001.Direct Anthelmintic effects of condensed tannins.

Caceres, A., Cano O, Samayoa B, Aguilar L. 1990. *J. Ethnopharmacol.* 38:55-73.

Chitravadivu, C., M. Bhoopathi, V. Balakrishnan, T. Elavazhagan and S. Jayakumar. 2009.

Antimicrobial Activity of Laehiums Prepared by Herbal Venders, South India, American eurasian J. Scientific res.4(3):142-147.

Deore S.L., and Khadabadi S.S. 2010. "In vitro anthelmintic studies of *Chlorophytum borivilianum* Sant. & Fernandez tubers" Indian .J Nat Prod Res. 1 (1): 53-56.

Dey P., Debnath P. and Bhakta T, 2012. "Evaluation of Anthelmintic Activity Of *Molineria Recurvata* Leaf Extracts" Int. Res J Pharm. App Sci. 2 (2): p. 16-20.

Ghosh, T., Maity, T.K., Bos, A. and Dash, G.K. 2005. Anthelmintic activity of *Bacopa*

monierri. Ind. J.Natural Product. 21(2): 16-19.

Gulnaz.A.R., and Savitha, G. 2013. Phytochemical evaluation of leaf and stem extracts of Siddha medicinal plant: *Sida cordata.* J. Evolution Med. Dental Sci. 2 (15): 2514-2521.

Gulnaz.A.R., and Savitha.G. 2013. Evaluation of Antimicrobial Activity of Leaf and Stem Extracts of Sidda Medicinal Plant *Sida cordata:* Inter. J. Med. Pharma. Sci. 3(3): 39-50.

Hoareau L. and Da Silva, E. 1999. Medicinal plants: A re- emerging health aid. E. J. B. 2(2): 56- 70.

Karale S.S., Godad A.P., Yashodhan B.W. and Suhas A.S, 2010. "Evaluation of in vitro anthelmintic activity of *Ceratophyllum demersum* Linn." Indian. Drug. 47 (8): 63-65.

Koppula S, Ammani K, Bobbarala V, and Bramhachari, P.V. 2010. Antibacterial activity screening of few medicinal plants from the southern region of India. J. Pharma. Res. 3: 2453-2456.

Kumar Ashok B.S., Lakshman K., Jayaveera K.N., Nandeesh R., Manoj B. and Ranganayakulu, D. 2010. "Comperative in vitro anthelmintic activity of three plants of Amaranthaceae family" Arch Biol Sci. 62 (1):185-89.

Lateef M., Iqbal Z., Khan M.N., Aktar M.S. and Jabbar A. 2003. "Anthelmintic activity of *Adhatoda vasica* roots" Int. J. Agri. Biol. 5 (1): 86-90.

Page A.P., and Winter, A.D. 2003. Enzymes involved in the biogensis of the nematode cuticle.53-85-148.

Panthi .M.P and Chaudhary, R.P. 2011 Antibacteriactivity of some selected folklore medicinal plants.

Suresh .G Arunachalam., N Senthil Kumar.(2011) J.Pharm. Res. 4(1): 283-284.

Thompson, D.P., and T.G.Geary. 1995. The structure and function of helminth surfaces in Biochemistry and Molecular Biology of parasites (j.j.marr, ed.), 1st ed. Academic press, New York, pp.203-232.

Waller, P.J., 1997. The global perspective of anthelmintic resistance in nematode parasites of sheep excluding Australasia. Proc. 4th Intl. Cong. Sheep Vet., Armidale, Australia, February 1997, pp: 59–63.

Waller, P.J., 1999. International approaches to the concept of integrated control of nematode parasites of livestock. Int. J. Parasitol. 29: 155–64.