International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-1, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Evaluation of *In-Vitro* Anthelmintic Potential of Plant Containing Hydrolysable Tannins

Pattewar AM[*1], Gundale DG[1], Dawalbaje AB[1], Shelge VB[2], Pekamwar SS[1]

[1*]*School of Pharmacy, S.R.T.M.U., Nanded, Maharashtra, India.*

[2]*Karnatka College of Pharmacy, Bidar, Karnatka, India.*

Manuscript No: IJPRS/V3/I1/00099, Received On: 24/02/2014, Accepted On: 04/03/2014

ABSTRACT

Number of medicinal plants plants having rich amount of condensed tannins are known to possess good anthelmintic potential and most of the published research are also based on anthelmintic potential of condensed tannins, but unfortunately the potential benefits of hydrolysable tannins has been neglected. So through this study we have evaluated the anthelmintic potential of two Indian medicinal plant as *Rhus chinensis* family *Anacardiaceae* and *Tamarix aphylla* family Tamaricaceae, having rich amount of hydrolysable tannins. All the collected well dried plant parts were extracted by using three different solvents as water, aqueous ethanol and aqueous acetone for three different techniques of extraction as maceration, soxhelation and simple percolation respectively. Freshly prepared extract were used for evaluating anthelmintic activity by using common methods of Nargud's with some minor modifications against two worms *Ascaris lumbricoides* and *Pheritema postuma*. Piperazine citrate was used as the standard. Study concludes that most of the extracts were found to possess reasonable anthelmintic in dose dependent manner.

KEYWORDS

Anthelmintic Activity, Hydrolisable Tannins, *Rhus Chinensis, Tamarix Aphylla, Ascaris Lumbricoides, Pheritema Postuma*

INTRODUCTION

The publication of case study ''This Wormy World'' by Norman Stoll in 1947 was the first warning to the world 'Helminthiasis' likely to become a growing cause of health damage especially in case of tropical countries; but at that time no one was in mood to consider helminthiasis as a serious cause of health damage and it was always considered as neglected health problem even after getting the another warning of resistance in 1970[1,2], when the first reports of anthelmintic resistance appeared. But in 1985 Waller published study on increasing health damage of helminthes infections and he warrant the world that "Drug resistance in helminthiasis: A real problem but not a merely a paper tiger"[2], Only 12 years later in 1997 once again the question was forwarded on serious problem of helminthes resistance by van Wyk 'How long before the drug resistance makes it impossible to control the Helminthiasis…?'[3,4] and today the helminthiasis becomes the one of the most common cause of ill health of peoples throughout the world especially in case of tropical countries suffering from problem of lack of sufficient sanitary and health facilities[5].

WHO recently estimates that the burden of ill health resulted due to helminthes infection is

***Address for Correspondence:**
Mr. Aditya M. Pattewar
School of Pharmacy, S.R.T.M.U., Nanded,
Maharashtra, India.
E-Mail Id: adi4ever@rocketmail.com

approximately equivalent to 50% of that of malaria and 25% of that of HIV and about 2.9 billion people are infected with one or more helminthes species and the condition is even more serious in case of China where about 63% of the people (707 million) among the population are infected with either one of the helminthes infection.[6,7] Ascariasis, Trichuriasis, Trichinosis, Strongyloidiasis, Filariasis, Ancylostomiasis, Enterobiasis, Onchocerciasis, Blindness are the most common and serious types of helminthes infections but the most devastating parasites of this group are hookworms, roundworm and whipworm each causing more than 1 billion infections annually. Filarial worm is also considered as dangerous especially in case of African countries as it causes elephantiasis and African river blindness. But amongst all this worms the hookworms is considered as most common cause of serious health damage as it causes anemia by feeding on blood capillaries directly. A hookworm approximately causes about 26.7 million annual deaths by causing iron-deficiency anemia in association with other diseases.[7,8]

So to control this huge health damage is of supreme importance and required integrated approach along with strategic and tactical use of available anthelmintics and careful management of grazing infections is recommended. However the problems related with the present anthelmintics like development of resistance, side effects and re-infection after discontinuation of therapy have created the question mark on the safety and liability of present anthelmintic to give complete cure from helminthiasis. In addition to this antigenic complexity of helminthes has created problem in the vaccine development.[1-3,9,10] For these several reasons the interest of researchers is diverted towards traditional medicinal plants known for their anthelmintic, antibacterial and insecticide uses.[11]

Number of medicinal plants has been reported for their anthelmintic use, especially plant having rich amount of condensed tannin are known to possess good anthelmintic potential and most of the published research are also based on anthelmintic potential of condensed tannins, but unfortunately the potential benefits of hydrolysable tannins has been neglected with no reason. So through this study we have evaluated the anthelmintic potential of two Indian medicinal plant as *Rhus chinensis* family *Anacardiaceae* and *Tamarix aphylla* family Tamaricaceae, having rich amount of hydrolysable tannins

Number of medicinal plants has been reported for their anthelmintic use to treat parasitic infections both in man and animals, plants like Karanjwa (Caesalpinia crista), Sohanjna (Morringa oleifera), Bakain (Melia azedarach), Kali-Zeeri (Vernonia anthelmintica), Babrung (Embellia ribes), Anar (Punica granatum) Dhak (Butea frondosa), Babchi (Psoralea corylifolia) are the some examples of commonly used anthelmintic plant.[12,15] This study was also forwarded with the same objective so as to find out, evaluate and to prove the anthelmintic activity of some Indian medicinal plants *Rhus chinensis* family *Anacardiaceae* and *Tamarix aphylla* family Tamaricaceae having rich amount of hydrolysable tannins which are often considered as neglected group phyto-constituents considering to their medicinal and therapeutic use.

MATERIALS AND METHOD

The plant parts used under study was dry galls of *Rhus chinensis* family *Anacardiaceae* and dry galls of *Tamarix aphylla* family Tamaricaceae. Dry galls of both the plant were purchased from the registered medicinal plant dealer and authenticated by liable personality. Collected galls were subjected to drying in to shade then ground in to powder form so as to make them ready for extraction.

The test parasites required for study were collected and authenticated from the Zoology, Department of Yashwantrao Chavan College, Karad, Dist – Satara. We had used *Ascaris lumbricoides* and *Pheritema postuma* as test parasite because *Ascaris lumbricoides* is often considered as common cause of intestinal infection while *Pheritema postuma* have anatomical and physiological similarities with

human parasite responsible for causing helminthiasis. Numbers of studies has reported the use of *Ascaris lumbricoides* and *Pheritema postuma* as model parasite to evaluate in-vitro anthelmintic activity.[16-18]

It has been reported that substances which have toxic effect against earthworms also have toxic effects against most of the worms by causing primary irritation which leads to paralysis to the worms that results in the withdrawal of the worm outside the body.[16-20]

Preparation and Preliminary Phytochemical Investigation of extract

Three extracts of plant were prepared by using three different solvents as water, aqueous ethanol (50%) and aqueous acetone (50%) for preparing aqueous, alcoholic and acetone extracts respectively. About 200g of powdered plant parts were used for extraction made by different method as per nature of solvent used.

Aqueous extract was made by double maceration, alcoholic extract by soxhelation while acetone extract was made by simple percolation. All the prepared extracts were collected and concentrated under the controlled temperature condition so as to obtain a powdered form of extract.

Whatever the amount of product obtained was considered as 100% and used for making further dilutions of different concentration by using distilled water as a solvent. The final dilutions were made and named as shown in table – 1.[21,22] Prepared extract were subjected to preliminary phytochemical investigation so as to get the rough guidelines about chemical composition of each extracts. Phytochemical extraction was made by performing primary chemical test and presences of respective chemical constituents in extracts were confirmed as shown in table – 2.[22]

Evaluation of Anthelmintic Activity

Method used for evaluation of anthelmintic activity was the common method for evaluation of anthelmintic activity as used in previous studies based on evaluation of anthelmintic activity.[16-20,24,25] First freshly prepared extracts were grouped as per table – 1, so as to evaluate activity of each extract separately. First extract was poured in to petri plates then worm (parasite/helminth) was placed in extracts under the continuous observation till its death. Meanwhile the observations were recorded as in the form of time required for first three attacks of paralysis and finally time required for the complete death of worms, which was confirmed by pointing with needle.

Table 1: Composition and Coding of Final Dilutions of Extract

Rhus chinensis Extract					
Aqueous Extract (A)		Aqueous Acetone (B)		Aqueous ethanol (C)	
Code	% Concentration	Code	% Concentration	Code	% Concentration
A1	10%	B1	10%	C1	10%
A2	25%	B2	25%	C2	25%
A3	50%	B3	50%	C3	50%
A4	100%	B4	100%	C4	100%
Tamarix aphylla Extract					
Aqueous Extract (D)		Aqueous Acetone (E)		Aqueous ethanol (F)	
D1	10%	E1	10%	F1	10%
D2	25%	E2	25%	F2	25%
D3	50%	E3	50%	F3	50%
D4	100%	E4	100%	F4	100%

Table 2: Preliminary Phytochemical Evaluation

Sr. No.	Phytochemical Test	Rhus chinensis Extract			Tamarix aphylla Extract		
		Aqueous (A)	Aqueous Acetone (B)	Aqueous Ethanol (C)	Aqueous (D)	Aqueous Acetone (E)	Aqueous Ethanol (F)
1.	Tannins	+	+	+	+	+	+
2.	Flavonoids	+	+	+	+	+	+
3.	Phenolic Compounds	-	+	+	-	+	+
4.	Terpinoids	-	-	-	-	+	+

Table 3: Observation and Result of Rhus chinensis Extract

Sr. No.	Sample Code	Pheritema postuma			Time Required for Death of parasite	Ascaris lumbricoides			Time Required for Death of parasite
		Time required for attack of paralysis				Time required for attack of paralysis			
		Ist	IInd	IIIrd		Ist	IInd	IIIrd	
1.	Controlled (Water)	-	-	-	-	-	-	-	-
2.	Piperazine Citrate (S)	1507	2212	2683	3190	1636	2378	2809	3268
3.	A1	3711	-	-	-	4137	-	-	-
4.	A2	2412	4331	5017	5422	2791	4801	5423	5914
5.	A3	1817	3048	3524	3832	2232	3461	3941	4316
6.	A4	1419	2124	2634	2792	1809	2432	3051	3329
7.	B1	3411	5011	-	-	3912	5811	-	-
8.	B2	2013	3582	4261	4607	2418	4008	4613	4982
9.	B3	1143	2232	2714	2913	1532	2608	3094	3324
10.	B4	768	1456	1746	1801	1214	1901	2213	2337
11.	C1	3112	4317	-	-	3711	5564	-	-
12.	C2	1856	3412	3937	4251	2307	3864	4436	4715
13.	C3	901	2018	2408	2617	1326	2321	2928	3111
14.	C4	557	1245	1509	1627	943	1532	1998	2143

RESULTS DISCUSSION

Preliminary Phytochemical Investigation

The preliminary phytochemical investigations of all prepared plant extracts were performed and presence of tannins, flavonoids, phenolics and terpenoids in prepared plant extract was confirmed. The result of phytochemical extraction was as shown in table – 2.

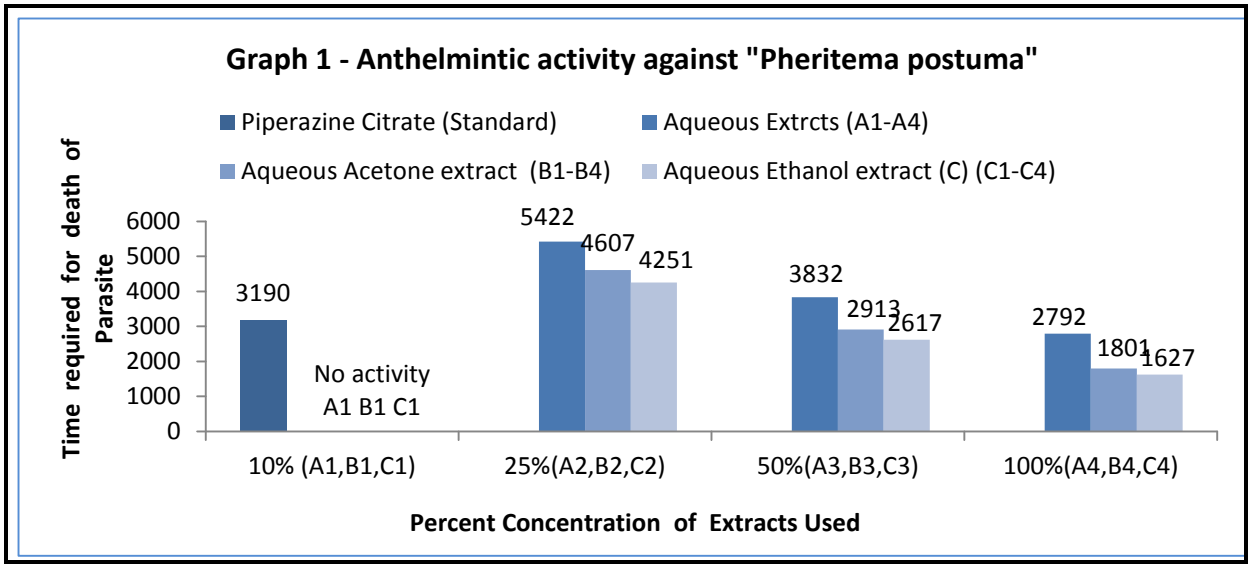
Evaluation of *In-Vitro* Anthelmintic Activity

Results were recorded as shown in table - 3 and 4 as time required for attacks of paralysis and finally the time required for the death of parasite. Mostly the death of parasite was occurred after three attacks of paralysis. At lower concentration range of 10% the death of parasite was not observed and it may be because of low potency; but further increase in concentration were showing good anthelmintic activity in dose dependent manner as shown in observation table. The extracts of 100% concentration were found to possess highest anthelmintic potency.

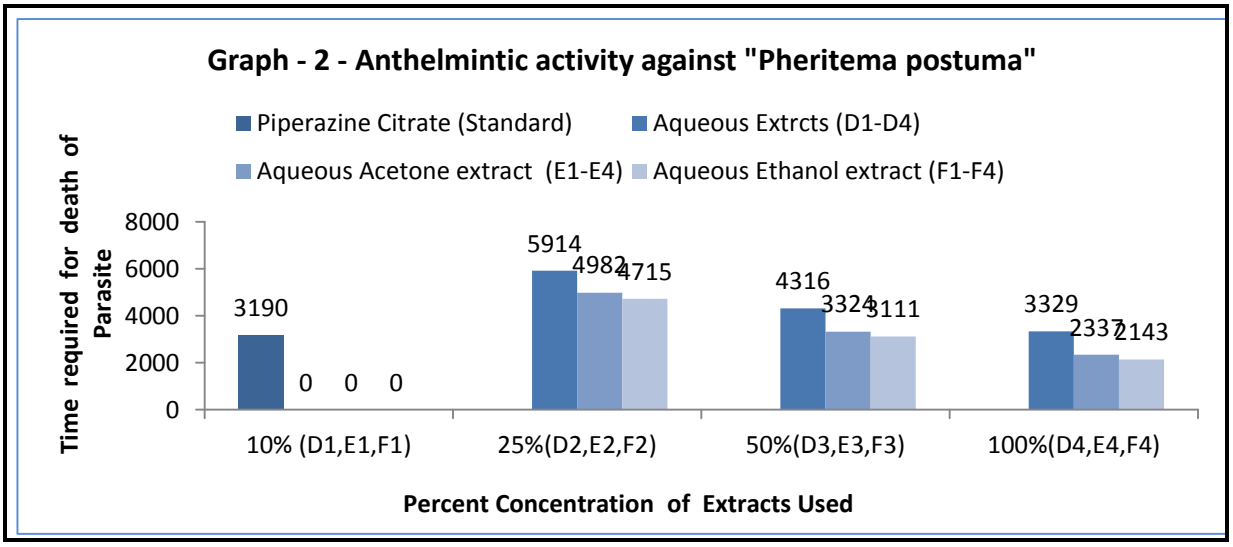
Results were also plotted as shown in graph for comparison of potency of both extracts.

Table 4: Observation and Result of Tamarix aphylla Extract

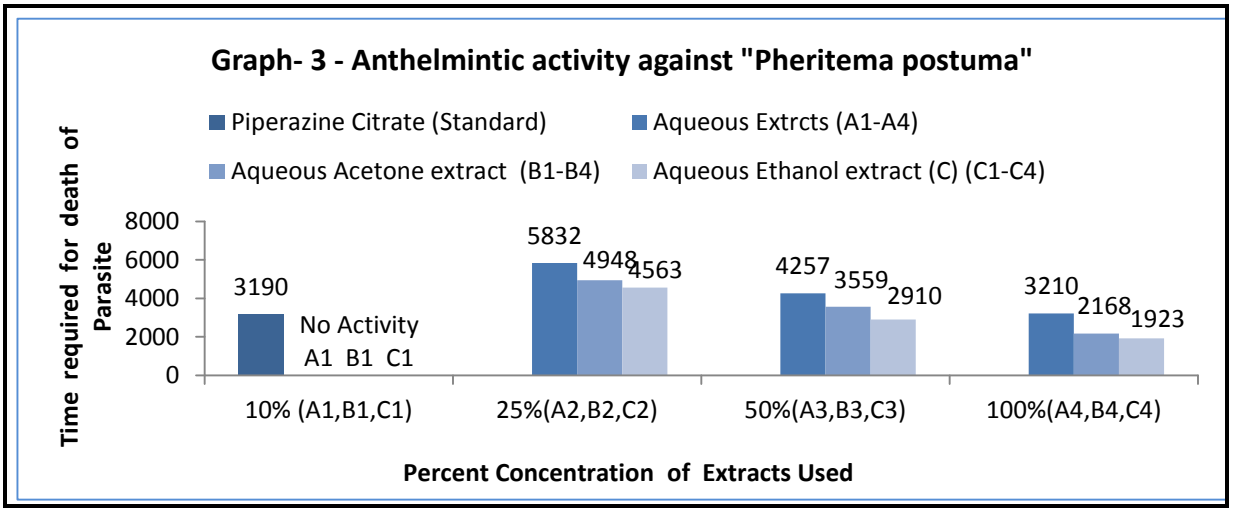
Sr. No.	Sample Code	Pheritema postuma			Time Required for Death of parasite	Ascaris lumbricoides			Time Required for Death of parasite
		Time required for attack of paralysis				Time required for attack of paralysis			
		Ist	IInd	IIIrd		Ist	IInd	IIIrd	
1.	Controlled (Water)	-	-	-	-	-	-	-	-
2.	Piperazine Citrate (S)	1507	2212	2683	3190	1636	2378	2809	3268
3.	A1	4127	-	-	-	5412	-	-	-
4.	A2	2832	4740	5423	5832	3214	5162	5370	6264
5.	A3	2198	3421	3942	4257	2786	3619	4297	4671
6.	A4	1796	2436	3051	3210	1168	2776	3397	3563
7.	B1	3762	5373	-	-	4423	6543	-	-
8.	B2	2359	3925	4611	4948	2732	4319	4927	5305
9.	B3	1489	2583	3056	3559	1839	2924	3409	3642
10.	B4	1126	1802	2116	2168	1521	2209	2521	2541
11.	C1	3418	4654	-	-	4123	6031	-	-
12.	C2	2159	3756	4349	4563	2554	4116	4688	4960
13.	C3	1208	2321	2714	2910	1326	2569	2984	3359
14.	C4	849	1558	1813	1923	1209	1798	2249	2399



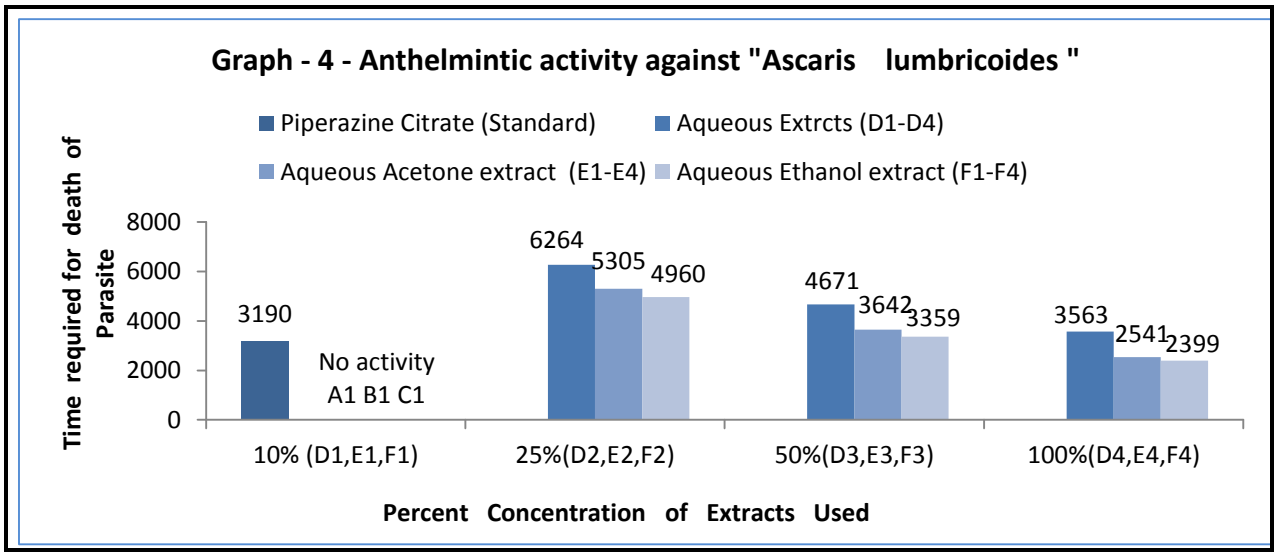
Graph- 1 - Comparison of anthelmintic activity of Rhus chinensis extracts against Pheritema postuma



Graph 2: Comparison of anthelmintic activity of Rhus chinensis extracts against Ascaris lumbricoides



Graph 3: Comparison of anthelmintic activity of Tamarix aphylla extracts against Pheritema postuma



Graph- 4 - Comparison of anthelmintic activity of Tamarix aphylla extracts against Ascaris lumbricoides

DISCUSSION

Both the plants were showing good anthelmintic potential in dose dependent manner. Plant *Rhus chinensis* was found more potent as compared to *Tamarix aphylla* against both the parasites. Bothe the plants were showing more potency against *Pheritema postuma* than *Ascaris lumbricoides*. The nature and choice of solvent used for extraction were also found to possess good impact on potency of extract, as aqueous ethanolic extract was found to possess highest potency fallowed by aqueous acetone extract fallowed by aqueous extract found to possess lowest potency. This effect may be due to variable amount of active chemical constituent extracted with different solvent used for extraction.

Phytochemical screening of extracts shows the presence of tannins, flavonoids, phenolics and terpenoids in *Tamarix aphylla* while terpenoids were absent in case of *Rhus chinensis.* The anthelmintic effect of plant was may be due to presence of chief amount of tannins, flavonids and phenolics present in plants, because in literature tannins, flavonoids as well as phenolics has been reported to possess potent anthelmintic effect.

Although tannin-rich plants are known to possess good anthelmintic activity, most of the published studies are based on evaluation of activity of condensed tannins.[26-30] At the same time while published literature lacks information about the therapeutic and medicinal importance including anthelmintic potential including nutritional benefits of plants containing hydrolysable tannins. Through this study we have evaluated the anthelmintic activity of two medicinal plants extracts containing hydrolysable tannins mostly.[27-30]

In vitro screening of plant for their potential anthelmintic activity may not correlate *in vivo* activity and toxicity profile of plants and although literature specifies that there is no correlation between in vitro and *in vivo* doses of plant extracts. The importance of *in vitro* testing lies in the fact that it permits the rapid assessment of plant extracts which can be used as candidate for *in vivo* testing.

CONCLUSION

Both the plants were found to possess good anthelmintic activity in doses dependent manner, aqueous-ethanolic extract of plant was found to possess highest potency fallowed by aqueous-acetone fallowed by aqueous extract, so study also reveals that the choice of solvent of extraction was also affecting the final result.

It is widely accepted that condensed tannins have very good therapeutic and nutritional profile, but at the same time the potential benefits of hydrolysable tannins have been neglecte. Our in vitro results put forward that plants containing Hydrolysable Tannins can possess good anthelmintic activity, so they should be considered seriously and must be tested for *in-vivo* activity, so as to promote their beneficial use in small ruminants.

Now it is well accepted that helminthiasis is one of the major cause of ill health of livestock especially in case of the tropical countries mostly including rest of world. So keeping in mind this huge burden of ill health the availability of commercial drugs may be inadequate. The phytochemical screening of medicinal plants fallowed by controlled anthelmintic trials through control strategies may offer increased possibility of getting effective and economical control measures of these parasitic diseases.

REFERENCES

1. Stoll, N. R. (1999). This wormy world. *The Journal of Parasitology*, 392-396.
2. Waller, P. J., & Anderson, N. (1985). Resistance to anthelmintics and the implications for animal production. *Resistance in Nematodes to Anthelmintic Drugs*, 1-11.
3. Van Wyk, J. A., Malan, F. S., & Randles, J. L. (1997). How long before resistance makes it impossible to control some field strains of Haemonchus contortus in South Africa with any of the modern anthelmintics?. *Veterinary Parasitology*, *70*(1), 111-122.
4. Van Wyk, J. A., Stenson, M. O., Van der Merwe, J. S., Vorster, R. J., & Viljoen, P. G. (1999). Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. *Onderstepoort Journal of Veterinary Research*, *4*, 273-284.
5. Lustigman, S., Prichard, R. K., Gazzinelli, A., Grant, W. N., Boatin, B. A., McCarthy, J. S., & Basáñez, M. G. (2012). A research agenda for helminth diseases of humans: the problem of helminthiases. *PLoS Neglected Tropical Diseases*, *6*(4), e1582.
6. Hotez, P. J., Ghosh, K., Hawdon, J., Narasimhan, S., Jones, B., & Bethony, J. (1997). Vaccines for hookworm infection. *The Pediatric Infectious Disease Journal*, *16*(10), 935-940.
7. Bethony, J., Brooker, S., Albonico, M., Geiger, S. M., Loukas, A., Diemert, D., & Hotez, P. J. (2006). Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet*, *367*(9521), 1521-1532.
8. Krogstad, D. J. (1993). Charting a course for the future: a society at the interface. *The American Journal of Tropical Medicine and Hygiene*, *48*(3), 303-312.
9. Waller, P. J., & Prichard, R. K. (1986). Drug resistance in nematodes. In *Chemotherapy of Parasitic Diseases* (pp. 339-362). Springer US.
10. Kaemmerer, K., & Buntenkötter, S. (1973). *The problem of residues in meat of edible domestic animals after application or intake of organophosphate esters*. Springer New York. 46, 1-13.
11. Satyavati, G. V., Raina, M. K., & Sharma, M., "Medicinal Plants of India", Vol. I. Indian Council of Medical Research, New Delhi; 1976, 201-206.
12. Nadkarni, A. K., Indian Materia Medica. 3rd ed. Bombay Popular Prakashan, Bombay, India. 1955.
13. Chopra, R. N. (1956). Glossary of Indian medicinal plants. *New Delhi, India;* 1956, 160-171.
14. Said, H. M. (Ed.). (1969). *Hamdard pharmacopoeia of eastern medicine*. Hamdard National Foundation. Karachi.
15. Akhtar, M. S., & Riffat, S. (1985). Anar – fruit-rinds against naturally acquired nematodal and cestodal infections. *Journal*

of Pharmacology of Punjab University of Lahor, 6, 17-24.

16. Gaind, K. N., & Budhiraja, R. D. (1967). Antibacterial and anthelmintic activity of Withania coagulans Dunal. *Indian Journal of Pharmacology, 29*, 185-186.
17. Kokate, D. K., & Varma, K. C. (1971). Anthelmintic activity of some essential oils. *Indian Journal of Hospital Pharmacy, 8*, 150-151.
18. Garg, S. C., & Siddiqui, N. (1992). Anthelmintic activity of Vernonia teres L. and Clerodendrum phlomidis L. *An annotated Bibliography of Indian Medicine, 11*, 1-3.
19. Nanda, A., Iyengar, M. A., Narayan, C. S., & Kulkarni, D. R. (1987). Investigations on the root bark of Aglaia odoratissima. *Fitoterapia, 58*, 189-191.
20. Sollmann, T. (1918). Anthelmintics: their efficacy as tested on earthworms. *Journal of Pharmacology, 1*, 129-136.
21. Pattewar, A. M., Patawar, V. A., Pandharkar T. M., & Yerawar, P. P. (2012). Evaluation of In-Vitro anthelmintic activity of Aconitum heterophyllum. *Journal of Chemical Biological and Physical Sciences, 2(4)*, 2401-2407.
22. Deore, S. L., Khadabadi, S. S., Kamdi, K. S., Ingle, V. P., Kawalkar, N. G., Sawarkar, P. S., & Vyas, A. J. (2009). In vitro Anthelmintic activity of Cassia tora. *International Journal of ChemTech Research, 1*(2), 177-179.
23. Kokate, C. K, Purohit, A., Gokhale, M. S. (1994). *Practical Pharmacognosy*, 10th ed. New Delhi, India: Vallabh Prakashan, New Delhi, 112-20.
24. Sarojini, N., Manjari, S. A., & Kanti, C. C. (2011). Phytochemical screening and anthelmintic activity study of Saraca indica leaves extracts. *International Research Journal of Pharmacy, 2*(5), 194-197.
25. Bendgude, R. D., Maniyar, M. G., Kondawar, M. S., Patil, S. B., & Hirave, R. V. (2012). Anthelmintic Activity of leaves of Mimosa pudica. *International Journal of Institutional Pharmacy and life sciences, 2*(1), 2249-6807.
26. Aremu, A. O., Fawole, O. A., Chukwujekwu, J. C., Light, M. E., Finnie, J. F., & Van Staden, J. (2010). *In vitro* antimicrobial, anthelmintic and cyclooxygenase-inhibitory activities and phytochemical analysis of Leucosidea sericea. *Journal of ethnopharmacology, 131*(1), 22-27.
27. Bate-Smith, E. C. (1962). The phenolic constituents of plants and their taxonomic significance. I. Dicotyledons. *Journal of the Linnean Society of London, Botany, 58*(371), 95-173.
28. Niezen, J. H., Waghorn, T. S., Charleston, W. A. G., & Waghorn, G. C. (1995). Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (Medicago sativa) or sulla (Hedysarum coronarium) which contains condensed tannins. *The Journal of Agricultural Science*, *125*(02), 281-289.
29. Athanasiadou, S., Kyriazakis, I., Jackson, F., & Coop, R. L. (2001). Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. *Veterinary Parasitology*, *99*(3), 205-219.
30. Thompson, D. P., & Geary, T. G. (1995). 12 The Structure and Function of Helminth Surfaces. *Biochemistry and Molecular Biology of Parasites*, 203-232.

