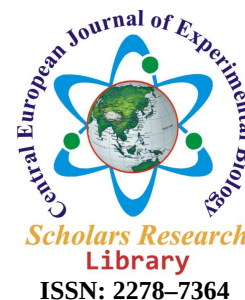




Scholars Research Library
Central European Journal of Experimental
Biology, 2012, 1 (3):100-106
(<http://scholarsresearchlibrary.com/archive.html>)



***In-vitro* anthelmintic activity of galenicals OF *Spermacoce ocymoides* (Burm F) DC**

^{*1}Pravat K Parhi, ²Prithwiraj Mohapatra

¹CMJ University, Shillong, India

²Indira Gandhi Institute of Pharmaceutical Sciences, Biju Pattnaik University of Technology, Rourkela, Odisha, India.

ABSTRACT

In the present investigation anthelmintic activity of the chloroform, methanol and aqueous extracts of the plant *spermacoce ocymoides* (Burm F) DC (Rubiaceae) were investigated as single extracts and in the different ratio of different extracts on adult Indian earthworm *Pheretima posthuma* group of Annelida. The chloroform extracts, methanolic extracts and aqueous extracts shown maximum anthelmintic activity in dose dependant manner at 35 mg/ml in ratio of 20 : 40 : 40 respectively which demonstrated paralysis of worms as well as death within 23.5 ± 2.6 minute and 44.33 ± 5.55 minutes respectively and found to be significant as compared to the standard drug albendazole (30 mg/ml) and piperazine citrate (15 mg/ml). Then suitable formulation was prepared as anthelmintic suspension and the anthelmintic activity of formulated suspension was evaluated on adult Indian earthworm *Pheretima posthuma* because they belong to same group of Annelida, family Megascolecidae as well as on worm parasites of human beings *Ascaridia galli* (nematodes) which are available in Chicken (*Gallus gallus*).

Key words: *Spermacoce*, anthelmintic, *Pheretima posthuma*, *Ascaridia galli*.

INTRODUCTION

Spermacoce ocymoides (Burm F) DC belongs to the family *rubiceae* is vascular plant without significant woody tissue above or at the ground. Forbs and herbs may be annual, biennial or perennial but always lack significant thickening by secondary woody growth and have buds borne at or below the ground surface, usually develops in loamy or sandy and slightly waterlogged soils. In East Africa it found in forest paths, leaf-litter, rocky paths in open grassland, It is found in Odisha and through out of India, also found in Cameroon, Congo, Gabon, Uganda etc as a wayside weed and it is also common in all America, also occurs in eastern Africa and East India [5].

From the literature review we got to know that the plant having several folklore and ethnomedicinal claims which includes: in Nigeria, the juice of the leaves is applied for ring worm and eczema and the sap is squeezed on to the wound or lesion [10]. The plant is used in hookworm and ringworm [18] infection. For treatment of microbial infections, Leaf of *Spermacoce ocymoides*, leaf of *Garcinia pictoria* and stem bark of *Syzygium cumini* are mixed, ground into a paste and heated with gingelly oil. The mixture thus obtained is applied topically on affected places to heal wounds [2]. The whole plant parts are used in diarrhea and dysentery [2]. Leaves are used in treatment of eczema and skin problems in Nizeria [10]. The plants are useful in curing gonorrhea, dysentery and skin diseases

[11]. For treatment of microbial infections Leaf of this plant, leaf of *Garcinia pictoria* and stem bark of *Syzygium cumini* are mixed, ground into a paste and heated with gingelly oil. The mixture thus obtained is applied topically on affected places to heal wounds [2]. The peoples of periphery of Balasore and Bhadrak district of Odisha are used the decoction of whole plant as Anthelmintic in children. Leaves of *Spermacoce ocymoides* and leaves of *Cassia tora* in together used in skin problems [2].

MATERIALS AND METHODS

Collection and Authentication of Plant:

The plant drug was collected from Nandan kanan area, Bhubaneswar, from the campus of Indira Gandhi Institute of Pharmaceutical Sciences, I.R.C village, Nayapalli, Bhubaneswar, and from the periphery of Balasore district, Odisha, India at morning hour and after noon in the month of February when the plant was in flowering stage.

The sample was identified to be *Spermacoce ocymoides* Burm F. DC (*Rubiaceae*) and the authentication certificate was issued by Dr. P.C Panda, sr. scientist, R.P.R.C, Bhubaneswar, Dr. Rashmi Prava Bahali, AYUSH Medical officer, Balikhand PHC(N), Balasore, Dr.Subhransu sekhar Mishra, Registered Ayurvedic medical practitioner, Mr. M. Rehman, Botanist, Department of Botany, Balangi college, Balasore of Odisha, India. After authentication the collected plant materials were shade dried at room temperature and then they are pulverized in mixer grinder to coarsely powdered drug and passed through sieve 60, stored in a well closed container by keeping away from direct sun light for the further use.

Preparation of extracts:

The plants *Spermacoce ocymoides* (Burm F) DC are shade dried at room temperature and pulverize by hand grinder. About 500 gm of the whole plant powder of *spermacoce ocymoides* was defatted with Petroleum ether (15-20°C) 72 hours, followed by successive extraction with Chloroform, Methanol and water respectively according to the increasing order of the polarity of the solvent. The respective extracts were dried. Standard methods were used for preliminary phytochemical screening of different extracts extract to know the nature of phyto-constituents present in it and the reagents for different qualitative test are prepared as per IP.

Preliminary Evaluation of Anthelmintic Activity of Different Extracts:

Drugs and chemicals used:

Drugs: Piperazine citrate (Glaxo smithkline) and Albendazole (Glaxo Smithkline), Chloroform extracts, Methanol extracts, aqueous extracts.

Chemicals: Sodium CMG, Normal saline.

Worms:

Indian adult earth worms (*Pheritima posthuma*) were collected from water logged areas of Aliha village, Balasore, Odisha and the worm was identified at the Department of Zoology, Balangi college (Under FM University), Balasore, Odisha and a authentication certificate was also provided from the departmental head.

Preliminary Evaluation of Anthelmintic Activity of Different Extracts:

The anthelmintic activity was evaluated on adult Indian earthworm *Pheritima posthuma* because these are resembled with human intestinal worms and both are belongs to same group of Annelida. The worms ranging from 6-8 cm long were selected for screening. Six groups of worms for each extracts, two groups of worm for standard-1 and standard-2, one group of worms for control were used to evaluate the anthelmintic properties of Chloroform, methanolic and aqueous extracts of *Spermacoce ocymoides*, Group 1-6 for each extracts were treated with 5, 10, 20, 25, 30 and 35 mg/ml of different extracts respectively (using 1% sodium CMG in normal saline), group 7-8 were treated with the drug albendazole (standard-1) and Piperazine citrate (standard-2) respectively in normal saline, group 9 with only normal saline as control. Each group included six worms. Observations were made for the time taken to set paralysis and death of the individual worms. Mean time for the paralysis in minute was noted when no movement of any sort could be observed and the time of death in minutes were counted and recorded from the very first second of introduction of worms in the respective drugs after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Albendazole (30 mg/ml), Piperazine citrate (15 mg/ml) was taken as reference compound. The activity of the test drugs are compared with the standard drugs. (Table.1)

Evaluation of Anthelmintic Activity of Different Extracts in Ratio:

The anthelmintic activity of the extracts in ratio were evaluated on adult Indian earthworm *Pheritima posthuma* as the method mentioned in preliminary evaluation of anthelmintic activity of different Extracts. Two groups of worms for each test drugs (extracts in ratio) were used to evaluate the anthelmintic properties of methanolic : aqueous extracts (50 : 50) as test 1 and Chloroform : methanolic : aqueous extracts (20 : 40 : 40) as test-2 of *Spermacoce ocymoides*. Group 1-2 were treated with 35 mg/ml each of test-1 and test-2 drug respectively (with 1% Sodium CMG in normal saline), each group included six worms. Observations were made for the time taken to set paralysis and death of the individual worms. Mean time for the paralysis in minute was noted when no movement of any sort could be observed and the time of death in minute counted and recorded from the very first second of introduction of worms in the respective drugs after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). The activity of the test-1 and test-2 was compared with the activity of reference standard Albendazole (30 mg/ml), Piperazine citrate (15 mg/ml) as previously observed from the preliminary evaluation of anthelmintic activity of different Extracts. (Table.2)

Formulation:**Materials:**

- a. Chloroform Extracts (700 mg)
- b. Methanol Extracts (1400 mg)
- c. Aqueous extracts (1400 mg)
- d. Sodium CMG (2% w/w)
- e. Propylene glycol (20% w/w)
- f. Calcium chloride (0.1% w/w))
- g. Methyl paraben (0.2% w/w)
- h. Propyl paraben (0.02% w/w)
- i. Purified water (q.s)

Method:

Chloroform, methanol and aqueous extracts were mixed together by triturating. The drugs are then wetted thoroughly with propylene glycol to reduce the interfacial tension [15]. The suspending agent, sodium CMG added in the aqueous medium containing methyl paraben, propyl paraben with continuous trituration and calcium chloride and then added into the wetted mass slowly with continuous trituration. The liquid vehicle (water) then added with constant trituration with the final suspension brought up to 100 ml and kept in a tightly closed container away from the light. (Table.3)

Evaluation of Anthelmintic Activity of Formulated suspension:**Worms:**

Indian adult earth worms (*Pheritima posthuma*) were collected from water logged areas of Aliha village, Balasore, Odisha and *Ascardia galli* (nematode) worm were collected from chicken (*Gallus gallus*) obtained from Suguna Poultry Pvt Ltd, Farm Code-46397. Both worm types were identified at the department of Zoology, Balangi College, FM University, Balasore, Odisha and a authentication certificate was also provided from the departmental head.

Drugs:

Piperazine citrate (Glaxo smithkline), Albendazole (Glaxo Smithkline), Formulated Suspension, Normal saline.

Method:

The anthelmintic activity of formulated suspension was evaluated on adult Indian earthworm *Pheritima posthuma* because they belong to same group of Annelida, family *Megascolecidae* as well as on worm parasites of human beings *Ascardia galli* (nematodes) which are available in Chicken (*Gallus gallus*) [1, 4 & 9]. Four groups of worms (for each worm type) were used to evaluate the anthelmintic activity of formulated product Spermacocy. Group 1 was treated with Formulated suspension (35 mg/ml), group 2-3 were treated with the drug albendazole (standard-1) and Piperazine citrate (standard-2) respectively in normal saline, group, 4 with only normal saline as control. Each group included six worms of each type. Observations were made for the time taken to set paralysis and death of the individual worms. Mean time for the paralysis in minute was noted when no movement of any sort could be observed and the time of death in minute counted and recorded from the very first second of introduction of worms in the respective drugs after ascertaining the worms neither moved when shaken vigorously nor when dipped in

warm water (50°C). The activity of the formulated suspension was compared with the activity of reference standard Albendazole (30 mg/ml), Piperazine citrate (15 mg/ml) (Table 4.)

RESULTS AND DISCUSSION

Preliminary phytochemical screening indicated the presence of oils and fatty substances in Petroleum ether, chloroform extracts indicated the presence of alkaloids, terpenoids, phyto sterols in, while methanolic extracts shown the presence of alkaloids, triterpenoid glycosides, cardiac glycosides, flavonoids, tannins and phenolic compounds and presence of reducing sugar, saponin glycosides, flavonoids, proteins and amino acids, tannins & phenolic compounds in the aqueous extracts. The petroleum ether extracts found to be no more phytochemicals other than oils and fats where as Chloroform, methanol and aqueous extracts found to have several phytochemicals. So, chloroform, methanolic and aqueous extracts were selected for the preliminary pharmacological screening to evaluate the anthelmintic activity of these three extracts on *in-vitro* model. The anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma* because they belong to same group of Annelida and due to their easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* [26, 19, 12, 1, 8, 21 & 20]. Albendazole (30 mg/ml), Piperazine citrate (15 mg/ml) was taken as reference standard. All the extracts shown the anthelmintic activity in dose dependent manner at 5 to 35mg/ml. The chloroform, methanolic and aqueous extracts at a concentration of 35 mg/ml revealed significant anthelmintic activity as compared to the standard drug piperazine citrate and albendazole as shown in Table 1. Out of three extracts Methanolic extracts showed maximum effect as compared to chloroform and aqueous extracts but the effect of aqueous and methanolic extracts at 35mg/ml are found to very close. Methanolic extracts and aqueous extracts demonstrated paralysis of worms in less time (22.16 ± 2 , and 23 ± 1.91 minute respectively) at a conc. of 35 mg /ml as compared to standard drug Albendazole (27.33 ± 2.31 minute) at the conc. Of 30 mg/ml and death of the worms demonstrated by the methanolic extract and aqueous extracts (35 mg/ml) within 48.16 ± 4.19 minute and 49.66 ± 4.52 minutes respectively whereas albendazole at the dose of 30 mg/ml demonstrated the death of the worms at 48.33 ± 5.07 minutes which was very similar to the effect of methanolic and aqueous extracts. Chloroform extract also showed a satisfactory paralytic value and death at 35 mg/ml as compared to standards. But piperazine citrate demonstrated paralysis of worms very quickly, that was at 8.16 ± 1.17 minute and death of the worms at 37.16 ± 2.41 minutes. Albendazole on the other hand causes death of the parasite. The lethal effect of Albendazole was attributed to its inhibition of tubulin polymerization and blocking glucose uptake [24] and Piperazine citrate causes flaccid paralysis of worms that resulting expulsion of worms by peristalsis. The presence of alkaloids, saponin glycosides or any other glycosides, flavonoids and tannins may be the responsible chemical constituents for demonstrating anthelmintic activity. Possibly phenolic compound [16] or saponin glycosides are responsible for the activity. The possible mechanism of phenolic compound may to interfere with energy generation by uncoupling oxidative phosphorylation or they may interfere with glycoprotein of cell surface because some synthetic phenolic anthelmintics e.g. niclosamide, oxclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is also possible that alkaloids present in the chloroform and methanolic extracts may act on central nervous system and caused paralysis of the *Pheritima posthuma* worms.

So, as because chloroform, methanolic and aqueous extracts showed significant anthelmintic activity at the concentration level 35 mg/ml and all the possible active constituents are present in these three extracts so it further decided to evaluate the extracts in different ratio at the concentration level 35 mg/ml on Indian earth worm *Pheritima posthuma*. Two types of preparatory extract ratio were decided to evaluate the anthelmintic activity before formulation. First one was Methanol : Aqueous extract with ratio 50:50 and second one was Chloroform : Methanol : water with ratio 20 : 40 : 40 respectively at concentration level 35 mg/ml.

Both the preparatory extract in ratio shown better anthelmintic activity than single extract as previously screened and tabulated. Methanol and aqueous extract in ratio 50 : 50 demonstrated paralysis of worms as well as death within 24.83 ± 3.36 minute and 46.66 ± 4.9 minutes respectively, whereas chloroform, methanol and aqueous extracts in ratio 20 : 40 : 40 with 35 mg/ml shown better anthelmintic activity than methanol and aqueous ratio 50: 50 as it caused paralysis of worms as well as death within 23.5 ± 2.6 minute and 44.33 ± 5.55 minutes respectively. The better activity of the chloroform, methanol and water extract in ratio may be shown due to the additive effect of phytoconstituents like alkaloid, saponin glycosides, flavonoids and phenolic compound as present in different extracts along with other phyto constituents.

Table 1: Screening of Anthelmintic Activity of Different Extracts

Drug	Concentration	Worm	
		<i>Pheritima posthuma</i>	
		Paralyzed Time in Minute	Death Time in Minute
Chloroform Extract	5mg/ml	57.5 ± 6.81	157 ± 5.39
	10mg/ml	45.66 ± 4.44	93.16 ± 4.27
	20mg/ml	42.5 ± 4.53	79.5 ± 4.74
	25mg/ml	42 ± 4.58	60.5 ± 5.92
	30mg/ml	39.5 ± 3.39	55.66 ± 4.64
	35mg/ml	32.83 ± 2.02	54.5 ± 6.07
Methanol Extract	5mg/ml	51.5 ± 5.97	130.66 ± 13.91
	10mg/ml	39.33 ± 3.97	95 ± 4.78
	20mg/ml	35.16 ± 3.82	71.83 ± 8.93
	25mg/ml	30.16 ± 3.75	56 ± 3.39
	30mg/ml	23.83 ± 2.37	50.5 ± 4.79
	35mg/ml	22.16 ± 2.00	48.16 ± 4.19
Aqueous Extracts	5mg/ml	55.16 ± 7.09	124 ± 7.97
	10mg/ml	48.16 ± 3.81	83.33 ± 9.70
	20mg/ml	39 ± 3.72	72.33 ± 6.40
	25mg/ml	32 ± 2.23	61.66 ± 5.50
	30mg/ml	27.33 ± 3.2	52.83 ± 4.12
	35mg/ml	23 ± 1.91	49.66 ± 4.52
Albendazole as Standard-1	30mg/ml	27.33 ± 2.31	48.33 ± 5.07
Standard-2 (Piperazine citrate)	15mg/ml	8.16 ± 1.17	37.16 ± 2.41

*Values in the table are mentioned in Mean ± SEM, n = 6

Table 2: Screening of Anthelmintic Activity of Different Extracts in Ratios

Drug	Concentration	Worm	
		<i>Pheritima posthuma</i>	
		Paralyzed Time in Minute	Death Time in Minute
Methanol : Water Extract (50:50)	35mg/ml	24.83 ± 3.36	46.66 ± 4.9
Chloroform: Methanol : Water Extract (20:40:40)	35mg/ml	23.5 ± 2.6	44.33 ± 5.55
Standard-1 (Albendazole)	30mg/ml	27.33 ± 2.31	48.33 ± 5.07
Standard-2 (Piperazine citrate)	15mg/ml	8.16 ± 1.17	37.16 ± 2.41

*Values in the table are mentioned in Mean ± SEM, n = 6

Table 3. Formulation of Galenical Suspension – 100ml (35mg/ml)

Ingredients	% w/w (on 3500 mg)	Amount (in mg)	Use
Chloroform Extracts	20%	700	Drug
Methanol Extracts	40%	1400	Drug
Aqueous extracts	40%	1400	Drug
Sodium CMG	2%	70	Suspending agent
Propylene glycol	20%	700	Wetting agent
Calcium chloride	0.1%	3.5	Divalent ion
Methyl paraben	0.2%	7	Preservative
Propyl paraben	0.02%	0.7	Preservative
Purified water	q.s	Up to 100ml	Vehicle

Anthelmintic activity of Formulated Suspension:

The anthelmintic activity of formulated suspension was evaluated on adult Indian earthworm *Pheritima posthuma* because they belong to same group of Annelida, family *Megascolecidae* as well as on worm parasites of human beings *Ascaridia galli* (nematodes) which are available in Chicken (*Gallus gallus*) and the *Ascaridia galli* is a suitable model for screening of anthelmintic drug was advocated earlier [1, 4, 9]. The formulated suspension revealed better activity than preliminary anthelmintic activity of extracts and extracts in ratio as were screened and observed before formulation. The formulated suspension demonstrated paralysis of worm *Pheritima posthuma* as well as death

within 21.16 ± 2.37 minute and 42.33 ± 4.9 minutes respectively whereas anthelmintic activity of formulated suspension shown highly significant on *Ascardia galli* with shortest time of paralysis and death like 8.33 ± 1.45 minutes and 21.5 ± 2.84 minutes respectively which was more effective than standard drugs Albendazole (30 mg/ml) and piperazine citrate (15mg/ml) as shown in table. Standard Albendazole at the concentration level of 30 mg /ml paralysed the worm *Ascardia galli* within 16.83 ± 2.63 minutes and death persisted within 32.66 ± 4.8 minutes where as standard drug piperazine citrate at the concentration level of 15 mg /ml paralysed the worm *Ascardia galli* within 8.16 ± 1.17 minutes and death persisted within 37.16 ± 2.41 minutes (Table no.4). The activity of the albendazole and piperazine citrate due to the reversible inhibiting neuromuscular transmission in the worm, probably by acting like GABA, the inhibitory neurotransmitter or GABA gated chloride channels in nematode muscle, which causes relaxation and depresses responsiveness to contractile action of Ach. Flaccid paralysis of the worms followed by death occur [24, 17]. The better activity may be shown in formulated suspension due to the additive effect of phytoconstituents like alkaloid, saponin glycosides, flavonoids and phenolic compound as present in different extracts along with other phyto constituents with proper dose dependant mixture of individual constituent made by the additives used in formulation.

In future research on this species of genus *Spermacoce* can be carry out by isolating the pure active principles.

Table 4: Screening of anthelmintic activity of formulated suspension

Drug	Conc.	Worms			
		<i>Pheritima Posthuma</i>		<i>Ascardia galli</i>	
		Paralyzed Time in Minute	Death Time in Minute	Paralyzed Time in Minute	Death Time in Minute
Formulated Drug	35mg/ml	21.16 ± 2.37	42.33 ± 3.63	8.33 ± 1.45	21.5 ± 2.84
Standard-1 (Albendazole)	30mg/ml	27.33 ± 2.31	48.33 ± 5.07	16.83 ± 2.63	32.66 ± 4.8
Standard-2 (Piperazine citrate)	15mg/ml	8.16 ± 1.17	37.16 ± 2.41	12 ± 1.65	42.33 ± 3.94
Control (Normal saline)	----	----	----	----	----

*Values in the table are mentioned in Mean $\pm$ SEM, n = 6

REFERENCE

- [1] EO Ajaiyeoba, P Onocha, and OT Olarenwaje, *Pharmaceutical Biol*, **2001**. **39**, 3, 217-220.
- [2] M Ayyanar, S Ignacimuthu **2009**, *Int. J Appl Res Nat Prod*, 2(3), 29-42.
- [3] EC Bate-Smith, *J Linn Soc Bot* **1962**, 58, 95-103.
- [4] Anindya Bose, HK Sundeep Kumar, Arundhuti Raut, Sujit Kumar Sahu and MBV Raju, *Journal of Basic and Clinical Pharmacy*, **2010**, 1, 2.
- [5] A Chiquieri, FR Di Maio, AL Peixoto, A distribuição geográfica da família Rubiaceae Juss. *Flora Brasiliensis de Martius*. Rodriguésia. **2004**, 55, 47–57.
- [6] EL Cabral, and Bacigalupo NM, Revision of Borreria Section Pseudodiodia Opera Bot Belg, **1996**, 7, 309- 327.
- [7] KD Chatterjee, Parasitology, Protozoology and Helminthology, Guha Ray Sree Saraswaty Press Ltd, Calcutta, **1967**, 168-169.
- [8] GK Dash, P Suresh, DM Kar, S Ganapathy and SB Panda, *J Nat Rem*, **2002**, 2 (2), 182-185.
- [9] SL Deore, SS Khadabadi, KS Kamdi, VP Ingle, NG Kawalkar, PS Sawarkar, UA Patil, AJ Vyas, *International Journal of ChemTech Research*, **2009**, 1, 2, 177-179.
- [10] RUB Ebana, BE Madunagu, ED Ekpe, IN Otung. *J Appl Bacteriol*, **1991**, 71, 398–401
- [11] Hutchinson and JM Dalziel, Cytogenetics of a mixed Brazilian population of Emilia sonchifolia. *Flora of Tropical West Africa*, **1963**, 2, British Crown Agent, London, 222-224.
- [12] ML Jain, SR Jain, *Planta Med*, **1972**, 22, 66-70.
- [13] RK Kaushik, JC Katiyar, AB Sen, *Indian J Med Res* **1974**, 62, 1367-75.
- [14] J Lal, S Chandra, V Raviprakash, Sabir M. *Indian Physiol Pharmacol* **1976**, 20, 64-68
- [15] L Lachman, HA Liberman, The Theory and Practice of Industrial Pharmacy, Verghese Publishing House, Bombay. **1990**, 3,
- [16] RJ Martin, Mode of action of anthelmintic drugs. *Vet J*, **1997**, 154, 11-34.
- [17] HP Rang And MM Dale, Text book of Pharmacology, Churchill Livingstone, Elsevier, **2007**, 6, 712.
- [18] S Shanmugham, *Journal of applied pharmaceutical sciences*, **1991**, 71 (05), 398-401.

-
- [19] T Sollamann, Anthelmintics: *J Pharmacol Exp Ther*, **1918**, **12**, 129-170.
- [20] YM Shivkar and VL Kumar, *Pharm. Biol.* **2003**, **41**, 263-265,
- [21] VD Szewezuk, ER Mongelli, AB Pomilio, *Molecular Med. Chem.* **2003**, **1**, 54-57.
- [22] V Tandon, P Pal, HS Roy, KS Reddy, *Parasitol Res*, **1997**, **83**, 492-8.
- [23] T Triratana, R Suwannuraks, W Naengchomnong, *J of Med Association of Tailand* **1991**, **74**, **5**, 283-87
- [24] KD Tripathi, *Essential of Pharmacology*, Jaypee Brothers Medical Publishers (P)LTD, **2008**, **6**, 808.
- [25] GW Thorn, RD Adams, E Braunwald, KJ Isselbacher, RG Petersdorf, *Harrison's Principles of Internal Medicine*. New York: McGraw Hill Co, **1977**.
- [26] RD Vidyarthi, *A Text Book of Zoology*. S. Chand and Co. New Delhi, **1967**, 329-370.
- [27] Sucheta A Gaikwad, Asha A. Kale, Balaso G. Jadhav, Nirmala R Deshpande and Jyoti P Salvekar, *Scholars Research Library, J of Nat Prod Plant Resour*, **2011**, **1** **2**, 62-66
- [28] Rasheed Ahmed, Ram Kumar Sahu, Krishna Kant Samele, Amit Roy, Jaya Dwivedi, *Scholars Research Library, Archives of Applied Science Research*, **2010**, **2**, **1**, 398-400.
- [29] T Venkatachalam, GRathinavel, V Kishor kumar1, P Kalaiselvi1, KG Lalitha and KL Senthilkumar, *Scholars Research Library, Der Pharma Chemica*, **2010**, **2**, **5**, 164-169.