

Research Article

Antifilarial activity of Methanolic extract of *Vitex negundo* L. leaves against *Setaria cervi* filarial parasite.

Keertisheel N Sahare*, Vinod Singh

Department of Microbiology, Barkatullah University, Bhopal- 462 026, (M.P.) India.

***Corresponding author**

Dr. Keertisheel N Sahare

Email: sahare_keerti@rediffmail.com

Abstract: Herbal medicinal practice is well known but largely observed all over the world. Currently available antifilarial drugs Diethylcarbamazine citrate, albandazole, Ivermectin and other combination of drugs are not effective against adult parasite. So adulticidal antifilarial drugs is required to control and management of the disease. In the present investigation, antifilarial activity was assessed for Methanolic extract of *Vitex negundo* L. Leaves against *Setaria cervi* filarial parasite. Activity was assessed by the method of motility inhibition and MTT reduction assay with concentrations 0.3-0.006 mgmL⁻¹ for 2 to 24 hrs incubation period respectively, by comparing with control. In motility assay, complete inhibition of motility was observed and in MTT reduction assay which gave >50% reduction for concentrations 0.06, 0.1 and 0.3 mgmL⁻¹ at 10, 6 and 2hrs incubation period respectively in a dose dependent manner (p<0.05). Antifilarial activity given by plant extract was found to be a function at their relative concentrations. Inhibitory concentration (IC50) for the plant extract was found to be 0.049mgmL⁻¹. *Vitex negundo* L. Leaves Methanolic extract showed significant reduction in adult motility in dose dependent manner contributes to the development of database and isolation of active molecule / principle for novel antifilarial drug candidate.

Keywords: Antifilarial activity, methanolic extract, *Setaria cervi*, *Vitex negundo* L.

INTRODUCTION

Herbal medicines constitute a major part in all traditional, Ayurveda and Unani system. World Health Organization has highlighted the development and utilization of medicinal plant resources in the developing countries so as to expand the health care to maximum number of population [1, 2]. Plant based medicinal constituents have massive therapeutic potential as they serve the purpose with lesser side effects that are often associated with synthetic antifilarials. Lymphatic filariasis is a chronic and debilitating tropical disease, with clinical manifestations like elephantiasis, Hydrocele and Lymphoedema. It is caused by vector borne parasites like *W. bancrofti*, *B. malayi* and *B. timori*. Approximately 120 million populations all over the world are suffering from filariasis.

The endemic drug in the treatment of filariasis for decades has been Diethylcarbamazine citrate (DEC) and Ivermectin is recommended in areas of Africa that are co endemic for Onchocerciasis. These drugs are active against microfilariae in interrupting transmission of the disease, but they are less effective against the adult worms [3, 4]. Adult parasite may survive for many years in the infected person [5] producing microfilariae and thereby facilitate transmission of the

disease through the mosquito vector to healthy Individual. Thus, removal of the parasite by means of microfilariae alone is extremely difficult. This warrants an effective and safe drug against the adult filarial worm. Plant *Vitex negundo* L. (*Verbenaceae*) is a reputed medicinal plant and its parts have been useful in Indian traditional system of medicine. This plant has shown many pharmacological activity such as: enzymes inhibition activity [6], anti-inflammatory activity [7], nitric oxide scavenging activity [8], anti-radical, anti-lipoperoxidative activity [9], CNS activity [10], hepatoprotective activity [11], antibacterial activity [12], larvicidal activity [13], anti-microfilarial [14], anti-fungal [15] and mosquito repellent activity [16]. Looking this encouraging advancement, in present study Methanolic extract of *Vitex negundo* L. leaves was screened *in vitro* for their possible antifilarial activity against *Setaria cervi* adult filarial parasite.

MATERIALS AND METHODS

Procuring plant material

Leaves of plant *Vitex negundo* L. were collected from the local areas of Bhopal (M.P.) and identified taxonomically by expert Botanist Dr Zia-Ul Hasan, Department of Botany, Safia Science College, Bhopal (M.P.). Specimen no. 410/Bot/Safia/2012. Voucher specimen was deposited in department, Plant

materials washed in tap water, shade dried and powdered.

Extraction

Leaves (1.5 kg) of *Vitex negundo* L. was extracted successively with petroleum ether (60°C - 80°C) (*Qualigens Fine Chem, Mumbai, SQ - grade*), CHCl₃ (*Ranchem, Mumbai, LR - grade*), ethyl acetate (*Merk India, Synthesis Grade*) and methanol (*Ranchem, Mumbai, AR - Grade*) by percolation method [17, 18].

Parasite

Adult *Setaria cervi* were obtained from the peritoneal cavity of freshly slaughtered cattle. The worms were washed repeatedly with normal saline (0.85%) to free them of any extraneous material and used for assay.

In-vitro motility inhibition assay

The worms were transferred immediately to DMEM (Dulbecco's modified eagle's medium) (*HiMedia, Mumbai, India*) with 0.01% Strepto-Penicillin (*HiMedia, Mumbai, India*) and supplemented with 10% heat-inactivated fetal calf serum (*HiMedia, Mumbai, India*). Dilutions of the extract of *Vitex negundo* L. were made in DMSO (*Dimethyl sulphoxide*) (*Merck India, drug use grade*) in such a way that 100 µL of which, when distributed to sterile disposable Petri dishes (35-mm diameter and 5-mL capacity) containing 3mL medium would give the required test concentration. Screening was done at concentrations ranging from 0.006 - 0.3 mgmL⁻¹. A simultaneous control was kept without the test solution but with 100µL DMSO in 3mL of the medium. Two worms (one male and one female) were introduced into each petri dish. Three replicates each were set up for both test and control. The worms were incubated at 37°C for 24 hrs in 5% CO₂ incubator and motility observed after 2 to 24 hrs. After exposure, the worms were washed twice with fresh medium and transferred to another set of fresh petri dish containing fresh medium without the test solution to find out whether any of the immotile worms regained motility in the 2 hrs post treatment period in drug free medium. If the worms did not revive, the condition was considered as irreversible and the concentration lethal. Each experiment was repeated three times [19].

MTT - reduction assay

Effect of the plant extract on adult female *Setaria* worms was studied by MTT (3-[4,5dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (*Hi-media, Mumbai, India*) - formazan reduction assay following the method described by Comely [20]. Because of the scarcity of male worms only female worms were used for these tests. The parasites were further incubated for 30 min individually in 0.5mL phosphate buffered saline (pH 7.4) containing 0.25 mgmL⁻¹ MTT. At the end of the incubation, worms

were carefully transferred to a microtiter plate containing 400 µL of DMSO (*Hi-media, Mumbai, India, Spectroscopic grade*) and allowed to be at room temperature for 1 hrs, with occasional gentle shaking to extract the color developed. The absorbance of the resulting formazan solution was then determined at 492 nm in an enzyme-linked immunosorbent assay reader (*LISA plus, Microtitre plate Reader*) relative to DMSO blank. High values of absorption correlate with high viability of the worms. Positive control was set up with adult females not treated with the test solution but exposed to DMSO as described in the above experiment. Adult worms that had previously been heat killed (56°C for 30 min) and incubated with MTT served as the negative control. Viability of the worms was estimated as percentage inhibition in formazan formation relative to solvent controls and heat killed worms [21] by following the formula:

$$\% \text{ inhibition (parameter)} = 100 - [(T - H) / (C - H)] \times 100$$

Where, T, C, and H are absorbance values obtained for the formazan produced in treated, control, and heat killed worms respectively.

Statistical analysis

For comparison of results between extract and respective controls, Student's *t* test was used. **P*<0.05 was considered as significant.

RESULTS

Plant extract

The solvent removed from the plant extract under reduced pressure from *Vitex negundo* L. leaves resulted in a semisolid residue.

In-vitro motility inhibition assay

Crude Methanolic extract was used for antifilarial screening against adults of the filarial worm *Setaria cervi*. Concentrations of 0.006 - 0.3 mgmL⁻¹ of the plant extract caused complete immobilization of the worms at 2 to 24hrs. exposure at 37°C, respectively, whereas in untreated control, all the worms were active (Table 1). Post exposure incubation in fresh medium (without test solution) for 2hrs did not revive the worms, confirming their death due to the effect of treatment. Thus, the results indicated that at higher concentrations, the inhibition in motility was faster, while at lower concentrations it was relatively slow.

MTT - reduction assay

The macrofilaricidal effect of the plant extract was further confirmed by comparing the treated worms to untreated control and heat-killed worms, in terms of MTT-formazan colorimetric assay (Table: 2). MTT is pale yellow in solution but when incubated with living cells is reduced by active mitochondria to yield dark blue formazan within the cells. During the assay, the formazan formed is extracted with DMSO and

quantitated colorimetrically. The very low absorbance value (0.319) observed for the heat-killed worms was due to the least production of formazan in dead worms. The percentage inhibition (>50%) considered significant, was found to be 59.5, 82.1 and 98.7% at concentrations 0.06, 0.1 and 0.3 mgmL⁻¹ at 10, 6 and 2hrs. incubation periods, indicating the significant effect of the plant extract at lower concentration.

Inhibitory concentration at which 50 per cent of the motility inhibition achieved (IC₅₀), was calculated by plotting the graph of percentage reduction in MTT – assay against different concentrations of herbal drug and the obtained values was 0.049 mgmL⁻¹. Both worm motility assay and MTT - reduction assay confirm the macrofilaricidal activity of the leaves extracts of *Vitex negundo* L.

Table 1: *In vitro* antifilarial activity of Methanol extract of *Vitex negundo* L. against adult filarial parasite in terms of motility inhibition.

Sample	Test concentration (mgmL ⁻¹)	Incubation time (end point) in hrs	Worm motility inhibition (Test)	Worm motility inhibition (Control)
Plant extract	0.006	24	#	†
	0.01	20	#	†
	0.03	14	#	†
	0.06	10	#	†
	0.1	6	#	†
	0.3	2	#	†

#Completely Immotile worm, †Completely motile worm

Table 2: *In vitro* antifilarial activity of *Vitex negundo* L. leaves Methanol extract against adult filarial parasite in terms of MTT reduction assay.

Sample	Incubation time (In hrs.)	Test concentrations (mgmL ⁻¹)	Absorbance at 492 nm (mean ± s.e.m.)	% reduction relative to solvent control ^C , heat killed ^H & treated worms ^T	IC ₅₀ (mgmL ⁻¹)
^C Control	24	-	1.009±0.03	-	0.049
^H Heat killed	0.5	-	0.323±0.028	-	
^T Plant extract	24	0.006	0.925±0.036	12.2	
	20	0.01	0.875±0.002*	19.5	
	14	0.03	0.786±0.001*	32.4	
	10	0.06	0.599±0.003*	59.5	
	6	0.1	0.443±0.008*	82.1	
	2	0.3	0.328±0.002*	98.7	

^C Positive control, ^HNegative control, ^TTreated worm with extract. P value represents the level of significance at **P* < 0.05 when comparing the mean value of absorbance observed for the formazan formed between treated and control worms.

DISCUSSION

Herbal medicines are being used by the world population mostly in the developing countries. These medicines are safe, suitable and compatible for human body with most likely lesser side effects [22]. Hence, WHO and TDR have recommended Ayurveda, Unani and Siddha system of medicine as holistic way [23].

In present research work, leaves methanolic extract of *Vitex negundo* L. was screened for antifilarial activity. This plant is a traditional medicinal plant used in many Ayurvedic drugs, shown significant antifilarial activity, against adult *Setaria cervi* filarial parasite. The treated worms were completely immobilized due to the lethal effect of the plant extract in a dose dependent manner at lower concentration. MTT reduction assay with treated extract confirmed its effect on the vitality of the worms by acting at the cellular stage, as signify by the reduced level of mitochondrial NAD(P)H-dependent cellular oxidoreductase enzyme that reduces the MTT to

formazan. The effects of this plant extracts shown in dose dependent manner. Consequently, inhibitory concentration (IC₅₀) was also calculated. Methanol extracts of *Vitex negundo* L. leaves showed promising activity. Antifilarial activity at lower concentration achieved in terms of reduction in adult motility as compared to the appropriate controls signify that this plant may have some active photochemical apply the actual therapeutic impact.

A study with aqueous and alcoholic extracts of the leaves of *Mallotus philippensis* (Lam.) against *Setaria cervi* reported antifilarial effect and also signify the importance of permeability factor [24]. Another antifilarial study was carried out to test the antifilarial efficacy of *Plumbago indigo/rosea* [25], Isolated Molecule from fruits of *Trachyspermum ammi*. [26], flowers extract of *Azadirachta indica* [27] and leaves extracts of *Excoecaria agallocha* L. [28] against *Setaria* filarial worm. These results indicate towards the importance of in depth study of *Vitex negundo* L. leaves

extract in terms of purification, isolation and identification of the active compound and study of antifilarial mechanism for enrichment of the antifilarial therapeutic range. This active potent therapeutic molecule may actually prove better in terms of cost effectiveness and patient fulfillment in combating this disease.

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REFERENCES

1. WHO; Neglected tropical diseases. Preventive chemotherapy and transmission control. WHO/CDS/NTD/2006,3a.
2. Goud PKK, Ramamurthy TP; Screening for antibacterial and antifungal activity of some medicinal plants of Nallamalais. Journal of Economic Taxonomy Botany, 2005; 29:704-708.
3. Ottesen EA, Duke BOL, Daram M, Behbehani K; Strategies and tools for the control/ elimination of lymphatic filariasis. Bulletin of World Health Organ, 1997; 75:491-493.
4. Noroes J, Dreyer, Santos A, Mendes VG, Medeiros Z, Addis D; Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti* *in vivo*. Transaction of Royal Society of Tropical Medicine and Hygiene, 1997; 91:78-81.
5. Vanamail P, Subramanian S, Das PK, Pani SP, Rajagopalan PK; Estimation of fecundic life span of *Wuchereria bancrofti* from longitudinal study of human infection in an endemic area of Pondicherry (South India). Indian Journal of Medical Research, 1990; 91: 293-297.
6. Azhar-Ul-Haq, Malik A, Khan MT, Anwar-Ul-Haq, Khan SB, Ahmad A, Choudhary MI; Tyrosinase inhibitory lignans from the methanol extract of the roots of *Vitex negundo* Linn. and their structure-activity relationship. Phytomedicine, 2006; 13:255-260.
7. Dharmasiri MG, Jayakody JR, Galhena G, Liyanage SS, Ratnasooriya WD; Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. Journal of Ethnopharmacology, 2003; 87: 199-206.
8. Jagetia GC, Baliga MS; The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants *in vitro*: a preliminary study. Journal Medical Food, 2004; 7: 343-354.
9. Munasinghe TC, Seneviratne CK, Thabrew MI, Abeysekera AM; Antiradical and antilipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection. Phytotherapy Research, 2001; 15:519-523.
10. Gupta M, Mazumder UK, Bhawal SR; CNS activity of *Vitex negundo* Linn. in mice. Indian. Journal of Experimental Biology, 1999; 37:143-146.
11. Avadhoot Y, Rana AC; Hepatoprotective effect of *Vitex negundo* against carbon tetrachloride-induced liver damage. Archives of Pharmacol Res, 1991;14:96-8.
12. Perumal Samy R, Ignacimuthu S, Sen A; Screening of 34 Indian medicinal plants for antibacterial properties. Journal of Ethnopharmacol, 1998; 62:173-182.
13. Pushpalatha E, Muthukrishnan J; Larvicidal activity of a few plant extracts against *Culex quinquefasciatus* and *Anopheles stephensi*. Indian Journal of Malariology, 1995; 32:14-23.
14. Sahare KN, Anandhraman V, Meshram VG, Meshram SU, Gajalakshmi D, Goswami K, Reddy MVR; *In vitro* effect of four herbal plants on the motility of *Brugia malayi*. Indian Journal of Medical Research, 2008; 127:467-471.
15. Damayanti M, Susheela K, Sharma GJ; Effect of plant extracts and systemic fungicide on the pineapple fruit-rotting fungus, *Ceratocystis paradoxa*. Cytobios, 1996; 86:155-165.
16. Hebbalkar DS, Hebbalkar GD, Sharma RN, Joshi VS, Bhat VS; Mosquito repellent activity of oils from *Vitex negundo* Linn. leaves. Indian Journal of Medical Research, 1992; 95:200-203.
17. Kokate CK; Practical Pharmacognosy. 4th edition, Vallabh Prakashan Delhi, 1993: 1-125.
18. Khandelwal KR; Textbook of Practical Pharmacognosy. 7th edition, Nirali Publication Pune, 2000: 1-189.
19. Murthy PK; Evaluation of two *in vitro* systems employing *Brugia malayi* parasite for prescreening of potential antifilarials. Current Science, 1999; 77:1084-1089.
20. Comley JCW, Rees MJ, Turner CH, Jenkins DC; Colorimetric quantification of filarial viability. International Journal of Parasitology, 1989; 19: 77-83.
21. Strote G, Bonow I, Kromer M, Tatjana Rubio de Kromer, Attah S, Opoku N; Chemotherapy for onchocerciasis: results of *in vitro* experiments with promising new compounds. Tropical Medicine International Health, 1998; 3:397-407.
22. Kamboj VP; Herbal medicine. Current Science, 2000; 78:35-39.
23. World Health Organization; The Promotion and Development of Traditional Medicine: Report of a WHO Meeting, WHO Technical Report Series, Geneva, 1978; 622.
24. Singh R, Singhal KC, Khan NU; Antifilarial activity of *Mallotus philippensis* Lam. on *Setaria cervie* (Nematoda: Filarioidea) *in vitro*. Indian Journal of Physiology and Pharmacology, 1997; 41:397-403.
25. Mathew N, Paily Abidha KP, Vanamail P, Kalyansundram M, Balraman K; Macrofilaricidal

- activity of plant *Plumbago Indica / rosea in vitro*. Drug Development Research, 2002; 56:33-39.
26. Mathew N, Misra-Bhattacharya S, Perumal V, Muthuswamy K. Antifilarial Lead Molecules Isolated from *Trachyspermum ammi*. Molecules, 2008;13:2156-8.
27. Mishra V, Parveen N, Singhal KC, Khan NU; Antifilarial activity of *Azadirachta indica* on cattle filarial parasite *Setaria cervi*. Fitoterapia, 2000;76;54-1.
28. Patra JK, Mahapatra AD, Rath S, Dhal NK, Thatoi H. Screening of antioxidant and antifilarial activity of leaves extracts of *Excoecaria agallocha* L. International Journal of Integrative Biology, 2009; 7:9-15.