

Antifilarial Screening of *Vitex negundo* L. Leaves Compound

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Abstract: Due to lack of a filaricidal drugs poses a challenge to filariasis elimination. *Vitex negundo* L. plant was used in traditional system of medicines in ancient time to treat the filariasis. In the present work, isolated compound tested *in vitro* against *Setaria cervi* filarial parasite and identified the possible oxidative role in culture supernatant of filarial parasite. The study was conducted for motility inhibition and MTT assays. Parameters of oxidative stress like MDA, Carbonyl content and Nitric oxide were identified. Compound isolated from *Vitex negundo* L. plant has revealed considerable antifilarial potential in *in vitro* system. Parameter levels of oxidative stress were calculated in dose dependent manners as compared to controls levels. The antifilarial activity was associated with oxidative mechanism was detected.

Index terms: Antifilarials, *In vitro*, oxidative stress, plant molecule, *Setaria cervi*.

I. INTRODUCTION

The filariasis becomes known throughout the world in tropical and subtropical countries. Filaria is transmitted by the mosquito vectors and disease infects by the parasitic *Wuchereria bancrofti* and *Brugia malayi* filarial nematodes (Liu LX, *et al.*). It is main public health problem, affected more than 100 million peoples throughout the world (WHO,

1987). Filariasis causes the deformity in prevalent areas and it is reason of a major socioeconomic and psychological cause. Approximate 45% of its 1 - billion Indian peoples lives in identified prevalent areas and 48 million population are affected (WHO, 2002), it is share for 40% disease burden globally (Das PK, *et al.*). Yearly loss calculated for filaria disease is up to a billion U.S. dollars as per the social and economic studies (Ramaiah KD, *et al.*).

World health organization has accepted filarial disease as main public health difficulty in the tropics and subtropics globally and specifically documented filaria in its TDR plan and launched a world agenda for eradication of filaria disease (WHO, 1987). The DEC, Albendazole and Ivermectin are popular anti-microfilarial drugs (Liu LX, *et al.*). They are not effective against the adult parasite. Adult parasite may survive in the human body for many years (Vanamail P, *et al.*). Due to these reasons, need an efficient and harmless drug to kill adult parasite.

Phyto drugs are important to treat various diseases and utilized in ayurvedic system in India. TDR scheme of WHO acknowledged herbal drug as main choice for potent drug resource. However, the major drawback to use the herbal medicine is due to lack of research validation. *Vitex* plant has given considerable anti-filarial potential and oxidative stress condition (Sharma RD, *et al.*). In this has plant identified many phenolic group containing compounds (Sahare KN, *et al.*, Díaz, F *et al.*). Polyphenolic group compounds are by nature antioxidants, isolated from plant source (Scalbert *et al.*). Some evidences shown that polyphenolic group containing flavonoid compounds have shown as pro-oxidants activity rather than anti-oxidants status (Fujisawa, S *et al.*). Oxidative stress mechanism is crucial in apoptosis (Buttke TM *et al.*).

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Looking above advancement, the present study was planned to screen antifilarial potential and possible oxidative action of isolated compound from *Vitex negundo* L. plant.

II. MATERIAL AND METHOD

A. Plant material:- *Vitex negundo* L. leaves of were collected from Bhopal (Madhya Pradesh) and taxonomic identification was carried out by Department of Botany, Safia Science College, Bhopal (M.P.).

B. Extraction of plant material:- Plant materials were extracted in petroleum ether, CHCl₃, Ethyl acetate and methanol (Kokate CK *et al.*, Khandelwal KR *et al.*). Isolation of active molecule was carried out by flash chromatography using Methanolic extract which have shown good antifilarial activity.

C. Parasite:- *Setaria cervi* adult parasites were collected from cattle (Slaughtered).

D. In vitro motility inhibition assay:- *S. cervi* parasites were taken (1 male + 1 female) in DMEM media containing Streptomycin - penicillin (0.01%) and heat-inactivated fetal calf serum (10%). Drug concentrations of compound were prepared in Dimethyl sulphoxide solution. Testing was done at 0.0005 - 0.02 mgmL⁻¹ concentration. Control was taken as DMSO. Plates were incubated at 37°C for 24 hours in CO₂ (5%) incubator and parasite motility was checked after 2 to 24 hours time interval. Further parasites were transferred in fresh DMEM culture media to check immotile parasite again becomes motile or not within 2 hours. If the parasite did not get back motile, the dose of compound was considered lethal for the parasite (Murthy PK *et al.* 1999).

E. MTT -assay:- Effect of purified compound on adult female *Setaria* worms was studied by MTT - farmazan reduction assay (Comely JCW *et al.*). Due to less availability of male parasite, only female parasites were taken. Parasites were incubated in 0.25 mgmL⁻¹ MTT solution, for 30 minutes. Further parasites were incubated in 400 µL of DMSO in gentle shaking condition to extract chromogen developed. O.D. was taken at 492 nm in an ELISA reader relative to DMSO blank. Higher O.D. values reflect to more viability of parasites. DMSO exposed females parasites were considered as positive control. Negative control heat killed (at 56°C) parasite incubated with MTT for 30 minutes was used.

Percentage inhibition in motility with reference to formazan formation was calculated (Strote G *et al.*) by formula-

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

Where T, C and H are O.D. values obtain in treated, control and heat killed worms.

F. MDA estimation:- Culture supernatants (0.5 ml), MDA (2.5 – 40 nM/ml) standards and 20% TCA (2.5 ml) + 0.67% Thiobarbituric acid (TBA) (1 ml) were taken and mixed. It is boiled in hot water bath up to 30 minute. Cooled and chromogen was extracted in n - butanol and organic phase was separated after centrifuge at 3,000 rpm. O.D. of standards and tests were taken at 530 nm. The concentration of MDA in test samples were calculated and expressed as nM MDA/ml (Satoh K *et al.*).

G. Protein Carbonilation Assay:- Protein carbonylation estimation was carried out by the method of Chakraborty. *et al.* The test samples were treated with 10% TCA to react with 10 mM DNPH (in 2M HCl) 1 hour. The pellet was centrifuged at 5000 rpm. Pallet washed three times with ethanol - ethyl acetate solution and dissolved in protein dissolving solution (2 gm SDS and 50 mg EDTA in 100 ml 80 mM L⁻¹ PBS at pH 8) and leave for 10 minutes at 37°C. O.D. was taken at 370 nm against 2 ML⁻¹ HCl. Carbonyl content was calculated by molar extinction – coefficient (21 X 10³ L Mol⁻¹ cm⁻¹).

H. Nitric Oxide Assay:- Nitric oxide levels in culture supernatants were estimated by the method of Waitumbi J *et al.* The nitric oxide in the 24hrs culture supernatants was estimated using Griess reagent. 100 µl Griess reagent was mixed in 100 µl of culture supernatant. O.D. was taken at 542 nm after 10 min incubation in ELISA reader. Nitric oxide levels in culture supernatant were calculated from standard graph of concentration 0.005 to 0.08 µM/ml.

I. Statistical analysis:- Results compared for test and controls. Student's t test was used. P < 0.05 was measured as significant value.

III. RESULTS AND DISCUSSION

A. Extraction of plant compound:- After removing the solvent, dry powder of compound obtained.

B. In vitro motility inhibition assay:- The isolated compound was screened for antifilarial potential against *Setaria cervi* 0.0005 to 0.02 mgmL⁻¹ concentration inhibit the total mobilization of parasites in 2 to 24 hours time interval. While all parasites were live in control plates (Table I). In motility inhibition assay at very low concentration, inhibit the complete motility as compare to control level.

Table I: *In vitro* antifilarial activity of plant compound in terms of motility inhibition.

Test conc. of compound (mgml ⁻¹)	Incubation time (end point) in Hrs	Worm motility inhibition (Test)	Worm motility inhibition (Control)
0.0005	24	#	†
0.001	20	#	†
0.002	14	#	†
0.005	10	#	†
0.01	6	#	†
0.02	2	#	†

#Completely Immotile worm, †completely motile worms.

C. *MTT – Formazan colorimetric assay*:- MTT assay confirmed the effect of compound. The formazan developed in DMSO was measured by ELISA reader. For heat - killed parasite the O.D. values 0.322 was recorded because of less formazan production in killed parasite. The percent inhibition >50% was measured considerable and it was 65.2, 80.9 and 97.7% at 0.005, 0.01 and 0.02 mgmL⁻¹ at 10, 6 and 2 hours incubation time, signifying the potent activity of compound (Table II). Concentration at 50% of inhibition in parasite mobility was measured at concentration 0.0032 mgmL⁻¹.

Table II: *In vitro* antifilarial activity of plant compound in term of MTT reduction assay.

Sample	Incubation time (Hrs.)	Test conc. (mgml ⁻¹)	Absorbance values at 492 nm (mean ± sem)	% reduction for solvent control ^c , heat killed ^h & treated worms ^t	IC 50 (mgml ⁻¹)
^c Control	24	-	1.006±0.02	-	0.0032
^h Heat killed	0.5	-	0.322±0.025	-	
^T Test.	24	0.0005	0.895±0.006*	14	
	20	0.001	0.811±0.006*	27.1	
	14	0.002	0.701±0.005*	44.8	
	10	0.005	0.565±0.006*	65.2	
	6	0.01	0.458±0.004*	80.9	
	2	0.02	0.327±0.134*	97.7	

^c Positive control, ^hnegative control, ^ttreated parasite. *P value corresponds to the levels of significance. $P < 0.05$

D. *MDA Estimation*:- Malondialdehyde is a secondary aldehyde degradation product of lipid peroxidation. MDA levels were checked in all culture supernatants. It is carried out by the customized method of Satoh K *et al.* The level of MDA (Table III) was calculated from the standard graph. The calculated MDA levels were found to be 0.6, 0.8, 1.5, 1.9, 2.6 and 3.2 for control it was 0.3nM/ml. The levels of MDA were achieved as ant filarial activity obtained (Fig. 1).

Table III: MDA, Carbonyl content and Nitric oxide levels in culture supernatants (nM/ml) of compound: Results shown are Mean ± SEM

Concentration of compound (mg / ml)	MDA levels (nm / ml)	Carbonyl content (nm / mg)	Nitric oxide levels (µm / ml)
0.0005	0.6 ± 0.055*	0.04 ± 0.04	0.006 ± 0.02*
0.001	0.8 ± 0.008*	0.11 ± 0.03*	0.010 ± 0.05*
0.002	1.5 ± 0.147*	0.28 ± 0.008*	0.022 ± 0.002*
0.005	1.9 ± 0.053	0.51 ± 0.007*	0.050 ± 0.02*
0.01	2.6 ± 0.149*	0.72 ± 0.024*	0.074 ± 0.002*
0.02	3.2 ± 0.149	0.95 ± 0.012*	0.093 ± 0.002*
Control	0.3 ± 0.054	0.03 ± 0.009	0.005 ± 0.003

*Significant when $p < 0.05$.

E. *Protein carbonilation test*:- Carbonyl contents were calculated 0.04, 0.11, 0.28, 0.51, 0.72, 0.95 nM/mg for control it was 0.03 nM/mg (Table III). The protein carbonilation levels were represented in nM/mg. Levels of Carbonyl content were calculated as anti-filarial activity achieved (Fig. 2).

F. *Nitric oxide Assay*:- Nitric oxide levels were checked in parasite culture supernatant and calculated on standard graph. (Table III). The levels of nitric oxide were represented in µM/ml. Nitric oxide levels 0.006, 0.010, 0.022, 0.050, 0.074 and 0.093 for control it was 0.005µM/ml calculated. Nitric oxide levels calculated as antifilarial activity was achieved (Fig. 3).

CONCLUSION

As per the WHO consent identification of potent antifilarial remedial drug is necessary because of enormous social and economic load of this disease in tropical and subtropical areas of world. In present research isolated the active compound from *Vitex negundo* L. leaves methanolic extract and we, achieved direct effect of this compound on filarial parasite. Levels of MDA, Carbonyl content and Nitric oxide, which is oxidative stress parameters, found as dose dependent antifilarial activity achieved. Thus, the results found for oxidative stress levels signify a close connection of oxidative and nitrosative relation in this filaricidal drug development study. Close relationship among oxidative stress parameter and inhibition in parasite viability over the specific drug concentration range indicate a basic cause of such oxidative stress of antifilarial compound. In similar study high antifilarial activity achieved at lesser concentration revealed considerable involvement of oxidative stress indicators with respective dose approach for filarial worms (Rachna, Sabharwal, Mahajan *et al.*). Close association found for Nitric oxide content in host defense and intracellular pathogens in

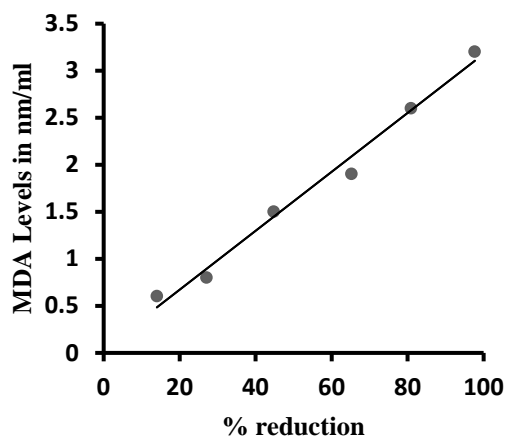


Fig.1: MDA Levels after 24 hours incubation in *in vitro* test. The levels were expressed in nm / ml.

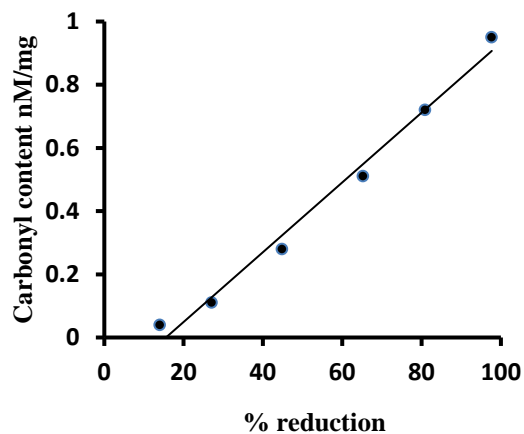


Fig. 2: Carbonyl content after 24 hours incubation in *in vitro* test. The levels were expressed in nM / mg.

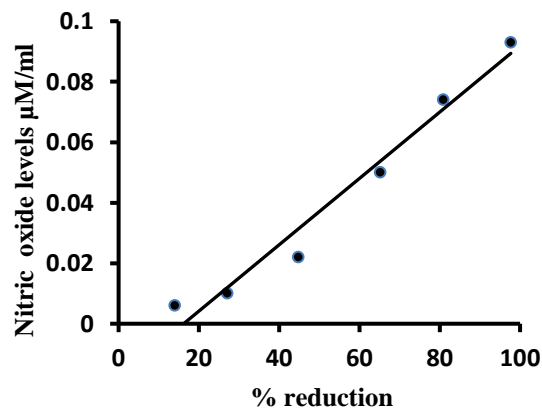


Fig. 3: Nitric oxide levels after 24 hours in *in vitro* test. The levels were expressed in µM/mL.

various studies (Rajan, TV *et al.*, Thomas, GR *et al.*). This antifilarial results exhibited the activity of isolated plant compound became nitrosative or oxidative mechanism. The isolated compound from *Vitex negundo* L. has revealed potential anti-filarial activity and probable mechanism identified as oxidative stress against *Setaria cervi* filarial parasite.

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