**In vitro** effect of four herbal plants on the motility of *Brugia malayi* microfilariae

K. N. Sahare, V. Anandharaman* V. G. Meshram**, S. U. Meshram, D. Gajalakshmi*  
K. Goswami* & M. V. R. Reddy*  

P.G. Department of Microbiology & Rajiv Gandhi Biotechnology Centre, RTM, Nagpur University, Nagpur  
*Jamnalal Bajaj Tropical Disease Research Centre & Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagrams & **Department of Biochemistry, Sindhu Mahavidyalaya Nagpur, India

Received December 11, 2006

**Background & objectives:** Disease burden due to lymphatic filariasis is disproportionately high despite mass drug administration with conventional drugs. Usage of herbal drugs in traditional medicine is quite well known but largely empirical. Hence the present study was designed to screen the **in vitro** antifilarial effect of four herbal plants on *Brugia malayi*.

**Methods:** Motility of microfilariae of *B. malayi* after incubation for 48 h with aqueous/methanol extracts of *Vitex negundo* L. (roots), *Butea monosperma* L. (roots and leaves), *Ricinus communis* L. (leaves), and *Aegle marmelos* Corr. (leaves) was explored in the concentration range of 20 to 100 ng/ml for possible antifilarial effect by comparing with suitable solvent control.

**Results:** *Butea monosperma* leaves and roots, *Vitex negundo* root and *Aegle marmelos* leaves showed significant inhibition of motility of microfilariae as compared to controls whereas inhibitory activity demonstrated by *Ricinus communis* L. leaves was not significant. Antifilarial effects imparted by all these extracts were found to be a function of their relative concentrations. Inhibitory concentrations (IC\(_{50}\)) for the plant extracts with significant antifilarial activity against *Brugia malayi* microfilariae in **in vitro** system have been derived to be 82, 83 and 70 ng/ml for *Vitex negundo* L., *Butea monosperma* L. and *Aegle marmelos* Corr. respectively.

**Interpretation & Conclusions:** The present study recorded significant antifilarial effect of all plant extracts studied except for *Ricinus communis* L. leaves and contributes to the development of database for novel drug candidates for human lymphatic filariasis.

**Key words** Antifilarials - *Aegle marmelos* Corr. - *microfilariae* - *Brugia malayi* - *Butea monosperma* L. - *Ricinus communis* L. - *Vitex negundo* L.
million disability adjusted life years (DALYs) and consequently launched a global programme for elimination of filariasis (GPELF). However, there are some serious limitations, such as lack of effective vaccines, potential threat of insecticide resistance against vector control methods, and the dearth in the repertoire of effective drugs.

The most widely employed drug in the treatment of lymphatic filariasis for decades has been diethylcarbamazine (DEC). Ivermectin is now recommended in certain areas of Africa that are co-endemic for Onchocerciasis. Although the existing drug of choice DEC is a good microfilaricide, it might have side effects. More importantly, owing to the lack of patients compliance coupled with failure in achieving targeted coverage (85%), use of this drug in mass drug administration strategy might not succeed in serving its purpose. Precisely because of these reasons, it is quite imperative to find out novel antifilarial drugs. The WHO has already outlined the nature of traditional medicine including herbal therapeutics. India has a rich tradition of practicing traditional herbal therapeutics in the form of Ayurveda, Unani and Siddha systems of medicine, which has got further fillip by the recent introduction of AYUSH scheme by the Government of India in an effort to integrate this legacy. However, therapeutic use of such herbal agents being largely empirical, establishment of the pharmacological effects in terms of modern scientific evidence based medicine is really a challenge. Hence it is essential to develop a large database of antifilarial herbal therapeutics. Experimental evidences of antifilarial activities of various such plants are coming up with the constant efforts made by the workers from different parts of the globe. To cite a few for example, ethanolic and aqueous extracts of Azadirachta indica on the cattle filarial parasite Setaria cervi, showed inhibitory effect against whole worm as well as the nerve muscle preparation of the organism and microfilariae. More interestingly such antifilarial effect has also been reported against human lymphatic filarial worm, Brugia malayi with crude extracts of green zoanthus and with Xylocarpus granatum, Tinospora crispa and Andrographis paniculata.

In the present study certain plants, which reportedly have significant anthelminthic/antifilarial effect in traditional usage, were screened in vitro for their antimicrofilarial activity against Brugia malayi. These included, Vitex negundo L. (Family: Verbenaceae), locally known as nirgundi found in warmer zone of India, Butea monosperma L. (Family: Fabaceae), local name palas or dhak, which is common throughout India, Ricinus communis L. (Family: Euphorbiaceae), commonly known as castor oil plant (or Erand), cultivated throughout hotter part of India and Aegle marmelos Corr. (Family: Rutaceae) known as bael.

**Material & Methods**

*Plant materials:* Vitex negundo L. (roots), Butea monosperma L. (leaves and roots), Ricinus communis L. (leaves) and Aegle marmelos Corr. (leaves) were collected from the local areas of Sausar, Chhindwara, Madhya Pradesh (India) and the same were identified in the P.G. Dept. of Botany, RTM, Nagpur University, Nagpur (Voucher specimen numbers 9022, 9024, 9025 & 9023 respectively).

*Preparation of herbal extracts:* The study was carried out at Department of biochemistry and JB Tropical Disease Research Centre, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha between January 2006 to June 2006. The leaves and roots of medicinal plants were kindly procured from RTM Nagpur University. They were washed, shade dried and powdered. The powdered form of Vitex negundo L. roots was extracted by 70 per cent ethanol (v/v) maceration followed by percolation using 70 per cent ethanol which produced 5.2 per cent (w/w) yield. Aegle marmelos Corr. Leaves were extracted by 70 per cent ethanolic extraction process with 3.7 per cent (w/w) yield whereas Ricinus communis L. leaves extract was prepared by methanolic extraction process which produced 4.7 per cent yield. Butea monosperma L. leaves and roots were extracted with double distilled water and further filtered and concentrated by keeping in hot air oven (40°C) to get semi solid residue which yielded 6.7 and 5.8 per cent (w/w) respectively. Extracts were stored in refrigerator for in vitro evaluation against Brugia malayi microfilaria using standard method.

*Parasites:* The Brugia malayi life cycle was established and maintained in jirds (Meriones unguiculatus) and mastomys (Mastomys natalensis) using mosquitoes (Aedes aegypti) as a vector by standard methods. Microfilariae (mf) were obtained by lavage of the peritoneal cavities of jirds with intraperitoneal filarial infection of 3 months or more duration. The mf were washed with RPMI 1640 medium (GIBCO laboratories, USA) (containing 20µg/ml gentamycin, 100µg/ml penicillin, 100µg/ml streptomycin) plated on sterile plastic petri-dishes and incubated at 37°C for 1 h to remove jirds peritoneal exudate cells. The mf were...
collected from petri-dishes, washed with RPMI 1640 medium and used for in vitro maintenance\textsuperscript{15}. The use of animals for the study was approved by the animal ethical committee of Mahatama Gandhi Institute of Medical Sciences, Sevagram.

In vitro screening for antifilarial activity: Crude extract of medicinal plants were diluted in suitable solvents like methanol / double distilled water to obtain the desired final concentration range (20-100 ng/ml) as previously optimized in our lab so as to obtain dose dependant effects against microfilariae in sterile 24 well culture plates (Nunc, Denmark) containing 900 µl of RPMI medium. Wells without any extract but with similar solvents in 900 µl of the medium were kept as corresponding controls. Approximately 100 microfilariae in 100 µl of RPMI medium were introduced into each well for every test samples and also for corresponding control samples (each individual samples in triplicates). The plates were incubated at 37°C for 48 h in CO\textsubscript{2} (5%) incubator. Mf motility was assessed by microscopy after 48 h of exposure (incubation for this time period was optimized during screening); the observations were recorded as the number of non motile mf out of all the 100 mf taken in each well for the study and represented as percentage (% reduction in Mf motility\textsuperscript{15}). All these conditions of assay procedure have been standardized in our laboratory to obtain reproducible results. Each experiment (in triplicate) was repeated thrice and results were represented as Mean ± SEM of per cent reduction in motility of three such observations.

Statistical analysis: For comparison of results between the extracts and respective controls, Student’s $t$ test was used. $P < 0.05$ was considered as significant.

| Table. In vitro effects of plant extracts on Brugia malayi microfilarial motility |
|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Percentage reduction in Mf motility by plants extracts | Percentage reduction in Mf motility by plants extracts | Percentage reduction in Mf motility by plants extracts | Percentage reduction in Mf motility by plants extracts | Percentage reduction in Mf motility by plants extracts |
| Concentration (ng/ml) | Vitex negundo L. (Roots) | Butea monosperma L. (Leaves) | Butea monosperma L. (Roots) | Ricinus communis L. (Leaves) | Aegle marmelos L. (Leaves) |
| 20 | 11.34 ± 2.11 | 13.68 ± 0.84* | 12.34 ± 0.56* | 6.76 ± 0.47 | 12.97 ± 0.84* |
| 40 | 16.36 ± 1.61* | 17.86 ± 0.37* | 13.94 ± 0.63* | 8.86 ± 2.20 | 15 ± 0.64* |
| 60 | 23.00 ± 1.13* | 25.8 ± 1.05* | 17.47 ± 1.55* | 9.95 ± 0.731 | 27 ± 2.62* |
| 80 | 44.87 ± 2.76* | 40.46 ± 6.08* | 24.97 ± 2.33* | 16.09 ± 2.28 | 73.98 ± 4.88* |
| 100 | 100* | 100* | 45.01 ± 3.18* | 21.18 ± 0.76 | 100* |
| Control 1 | 7.86 ± 0.89 | - | - | - | 7.86 ± 0.89 |
| Control 2 | - | - | - | - | - |
| Control 3 | 4.76 ± 1.07 | 4.76 ± 1.07 | - | - | - |

Results shown are mean ± SEM of per cent reduction in motility except where 100 per cent effect was observed

* $P < 0.05$ when compared with respective control levels (Control 1- RPMI+70 per cent ethanol, Control 2- RPMI +70% methanol, Control 3- RPMI + double distilled water)

Results

The plants extracts were screened for antifilarial activity against microfilariae of Brugia malayi. Of the five different extracts tested, aqueous extract of Butea monosperma L. (leaves and roots), ethanolic extract of Vitex negundo L. (roots) and of Aegle marmelos Corr. (leaves) showed significant loss of motility in a dose dependent manner as opposed to respective controls. Particularly, extracts of Vitex negundo L., Aegle marmelos Corr. and Butea monosperma L. showed 100 per cent loss of motility of microfilarial parasites at 100 ng/ml concentration. However, with water extract of Butea monosperma L. and 70 per cent methanolic extract of Ricinus communis L. the observed loss of microfilarial motility was not as high as those found with the other extracts used in this study (Table).

Inhibitory concentration at which 50 per cent of the total mf lost motility (IC\textsubscript{50}), was calculated for the extracts with high antifilarial activity (100% loss of mf motility) by plotting the graph of percentage motility of the parasite against different concentrations of extracts and the obtained values were 82, 83 and 70 ng/ml for ethanolic extract of Vitex nigundo L. (roots), aqueous extract of Butea monosperma L. (leaves) and ethanolic extract of Aegle marmelos Corr. (leaves) respectively.

Discussion

Considering the huge socio-economic encumber of filarial disease on the developing countries, where this disease is much more preponderant, in accordance to WHO/TDR mandate, detection and establishment of novel antifilarial therapeutic candidates emerged up as a necessity.
Herbal medicines are quite popular and being used by about 80 per cent of the world population mostly in the developing countries. These are time-tested for their safety, efficacy, and cultural acceptability. The chemical ingredients of these plants are believed to have better compatibility with the human body with presumably lesser side effects\textsuperscript{18}. Hence, very aptly the WHO has referred this system of medicine as holistic approach towards health\textsuperscript{19}. A growing body of evidence assembled from previous studies identified antifilarial activities of various herbal medicines\textsuperscript{20}. The present work is an attempt to contribute to this database by screening of crude plant extracts for antimicrofilarial activity on \textit{Brugia malayi}. All the extracts showed their antifilarial effects in a dose dependent manner; however aqueous extract of \textit{Butea monosperma} L., ethanolic extracts of \textit{Vitex negundo} L. and \textit{Aegle marmelos} Corr. showed significant results. The observed antifilarial effects of these three plant extracts recorded in terms of loss of motility in comparison to the suitable controls indicate that these can be considered as potential drug candidates, though this should be further confirmed by studying corresponding actual loss of viability of the parasites. Consequently, inhibitory concentration (IC\textsubscript{50}) was also calculated. Ethanolic extracts of \textit{nirgundi} roots and \textit{bael} leaves showed promising result while \textit{palas} leaves but not the roots in water showed significant efficacy. These results indicate that certain active compounds present in these extracts exert the actual therapeutic impact depending on their solubility and/or permeability properties. Hence, it would be interesting to find out the rationale behind the pharmaceutical efficacy of these potential drug candidates in the light of phytochemical analysis of these extracts. A study with aqueous and alcoholic extracts of the leaves of \textit{Mallotus philippensis} (Lam.) against \textit{Setaria cervi} reported antifilarial effect and also highlighted the importance of permeability factor\textsuperscript{21}. Another study was carried out to test the antifilarial efficacy of \textit{Cardiospermum halicacabum} against related filarial worm \textit{Brugia pahangi}\textsuperscript{22}.

In conclusion, our findings indicate towards the importance of in depth study of these herbal drugs for enrichment of the antifilarial therapeutic repertoire; these traditional therapeutic alternatives may actually prove better in terms of cost-effectiveness and patient compliance in combating this disease.

Acknowledgment

Authors thank Dr Alka Chaturvedi, Reader, P.G. Dept. of Botany, RTM, Nagpur University, Nagpur, India for identification and authentification of plant species. This work was supported by the research project grants from Department of Biotechnology (DBT), New Delhi.

References

5. WHO in the South-East Asia region: 38\textsuperscript{th} meeting of the Consultative Committee for Programme Development and Management (CCPDM). 2002; www.searo.who.int/en
section1430/section1439.
13. Franssen FF, Smeijsters LJ, Berger I, Medinilla Aldana BE. \textit{In vivo} and \textit{in vitro} antiplasmodial activities of some plants traditionally used in Guatemala against Malaria. \textit{Antimicrob Agents Chemother} 1997; 41 : 1500-3


Reprint requests: Dr Kalyan Goswami, Associate Professor, Department of Biochemistry, JB Tropical Disease Research Centre, Mahatma Gandhi Institute of Medical Sciences, Sevagram - 442 102, India
e-mail: goswamikln@gmail.com, goswamikln@rediffmail.com