IN VITRO MACROFILARICIDAL ACTIVITY OF TRADITIONAL MEDICINAL PLANTS

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ABSTRACT

Lymphatic filariasis is the second leading cause of permanent and long term disability in the world. The most widely used drug in the treatment of Lymphatic filariasis is Diethylcarbamazine, Invermectin and Albendazole. These drugs have marked activity against microfilariae and are much less effective against adult worm. This warrants the need for developing an effective and safe drug, which could kill or sterilize the adult worm. Cassia occidentalis Linn, Oldenlandia herbacea L.Roxb, Sida acuta Burm.f, Clitoria ternatea Linn and Euphorbia hirta Linn were selected for the present study. Adult Setaria digitata, a filarial parasite of cattle were used as the test organism for screening the macrofilaricidal activity of the selected plants. Methanolic extract of medicinal plants were used for the study. Worm motility assay was performed and all the plant extracts showed complete immobilization of worms at 10 mg/ml at the end of 4 hours incubation period. MTT formazan colorimetric assay for viability of worms were carried out. Cassia occidentalis, Oldenlandia herbacea and Sida acuta exhibited > 50 % of formazan inhibition at 4 hours. Clitoria ternatea and Euphorbia hirta exhibited <50 % of inhibition at 4 hours. Oldenlandia herbacea exhibited 65.67, 78.6 and 85.66 % of inhibition for the concentration of 1, 5 and 10 mg/ml respectively. The study identified Oldenlandia herbacea as a potential macrofilaricidal agent.
KEYWORDS: Lymphatic filariasis, macrofilaricidal, *Cassia occidentalis*, *Oldenlandia herbacea*, *Sida acuta*, *Clitoria ternatea*, *Euphorbia hirta*.

INTRODUCTION

Lymphatic filariasis is the second leading cause of permanent and long-term disability in the world. Diethylcarbamazine, Ivermectin and Albendazole are the most widely used drugs in the treatment of Lymphatic filariasis. These drugs have marked activity against microfilariae but they are much less effective against adult worm. None of these is effective in killing the adult worms, which can live in the host for several years and the treatments are therefore aimed solely at reducing transmission and pathology. Plant-based drugs plants to treat Lymphatic filariasis effectively have not been developed. This warrants the need for developing an effective and safe drug, which could kill or sterilize the adult worm.\(^1\) Three nematode parasites causing Lymphatic filariasis in humans are *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. *Wuchereria bancrofti*, transmitted by the ubiquitous vector, *Culex quinquefasciatus* has been the predominant infection contributing to 99.4% of the problem in the country.\(^2\)

From time immemorial man has been using plants as food and medicine. Today, about 50% of drugs used in modern medicine are of plant origin.\(^2\) Several medicinal agents derived and developed from plants and used in traditional medicinal systems such as Ayurveda, Unani, Siddha and others. Secondary metabolites produced by plants constitute a rich source of bioactive substances and currently the scientific interest has increased due to the search for new drug from plant origin.\(^3\) The majority of drugs active against infectious agents are derived from natural products. Potent antimalarial drugs, Quinine (an alkaloid isolated from the bark of Cinchona), Chloroquine (a synthetic analogue of quinine), Artemisinin (isolated from the Chinese medicinal plant *Artemisia annua* L), artemether (a semisynthetic derivative of Artemisinin with less side effects), and an antihelmintic, Ascaridol is, derived from *Chenopodium ambrosoides* and the microfilaricide Ivermectin (semisynthetic derivative of avermectins isolated from *Streptomyces avermitilis*) demonstrate that natural products can provide efficient antiparasitic agents.\(^4\)

MATERIALS AND METHODS

Identification and authentication of plant

*Cassia occidentalis* Linn, *Oldenlandia herbacea* L.Roxb, *Sida acuta* Burm.f, *Clitoria ternatea* Linn and *Euphorbia hirta* Linn plants have been traditionally used for Filariasis,
anthelminitic, worm infestation, testicular swelling, elephantiasis, and were collected from places in and around Trichy, identified and authenticated with the specimen at RAPINAT herbarium St. Joseph’s College, Trichy, Tamilnadu. (Voucher specimen number-S001, S002, S003, S004 and S005 respectively).

**Preparation of methanolic plant extract**
Aerial parts of the selected plant were washed, shade dried and coarsely powdered with help of electrical blender. About 100 gm of the powdered sample was used for extraction with 750 ml of analytical reagent (AR) grade methanol in a sox let apparatus for 5 hours. After extraction the solvent was flash evaporated in a rotary vacuum evaporator, the residue was weighed and reconstituted in ethyl alcohol and used for the study.

**Preparation of experimental organisms**
Adults cattle filarial parasite *Setaria digitata* (Nematoda: Filarioidea) were used as the test organism for screening the macrofilaricidal activity. Adult *S.digitata* worms were collected from the peritoneal cavity of freshly slaughtered cattle and used for the experiment within an hour. The worms were repeatedly washed with normal saline (0.85%) to make them free from any extraneous material and transferring to Dulbecco’s Modified Eagle Medium (DMEM) containing 0.01% Strepto-penicillin and supplement with 10% heat inactivated foetal calf serum.

**Worm Motility Assay**
Three ml of DMEM was poured to sterile disposable Petri dishes. Dilutions of the plant extracts were made in ethyl alcohol. Screening was done at concentrations ranging from 1 – 10 mg ml\(^{-1}\) of the crude extract. A simultaneous control was maintained with ethanol. Two adult worms were in introduced into each petri dish. Six replicates for both test and control were maintained. The worms were incubated at 37\(^0\)C one hour and four hours. After one and four hours the number of immobilized worms in petri dish was counted. Immediately after counting the worms were washed twice with fresh medium and transferred into another set of petri dishes containing fresh medium, without test solution to test for immobility and regaining of motility after 1, 6, and 12 hours post exposure. If the worms did not regain motility the condition was considered as irreversible and the concentration lethal.

**MTT- Formazan Colorimetric Assay**
Plant extracts in different concentration by motility assay were further screened for viability
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of adult through 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) reduction assay. After the exposure of the worms to various concentrations (1.5 and 10 mg/ml) of each effective crude extract in DMEM, the parasites were further incubated for 30 min individually in 0.5 ml phosphate buffered saline (pH 7.4) containing 0.25 mg /ml of MTT. At the end of the MTT incubation, the worms were transferred to a microtitre plate containing 400 μl of spectroscopic grade dimethyl sulphoxide (DMSO) and allowed to be at room temperature for 1 h, with occasional gentle shaking to extract the colour developed. The absorbance of the resulting formazan solution was then determined at 492 nm. Viability of the worms was estimated as percentage inhibition in formazan formation relative to solvent-control and heat-killed worms.

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\% \text{ Inhibition (parameter)} = 100 - \left[ \frac{(T - H)}{(C - H)} \right] \times 100
\]

where T, C and H are absorbance values obtained for the formazan produced in treated, control and heat-killed worms, respectively. [4]

RESULTS

The worm motility assay showed that three plant extracts (Cassia occidentalis, Oldenlandia herbcea, Sida acuta) exhibited macrofilaricidal activity at concentration below 3mg/ml at an exposure period of 4 hours. Oldenlandia herbcea and Sida acuta exhibited macrofilaricidal activity within 1 hour at a dose level of 8 mg/ml. Cassia occidentalis and Sida acuta showed complete suppression of motility within 4 hours at a dose level of 2 mg/ml.

Clitoria ternatea and Euphorbia hirta were effective as the worms were inactive till end of the test period at concentration ranging from 6-10 mg/ml at 4 hours. Cassia occidentalis, Clitoria ternatea, and Euphorbia hirta extracts treated worms exhibited a rapid flaccid paralysis at lower test concentrations, but regained the movement progressively during post exposure period. The entire control worms were active and alive till the end of the test period. All the plant extracts showed complete immobilization of worms at 10 mg/ml at the end of 4 hours incubation period.

The plants selected for the present study can be considered to be macrofilaricidal as the results depict the immobilization of the worms when treated with the plant extracts. It is a clear qualitative picture of the viability of the worms in comparison with control groups.
The results of percentage reduction in formazan formed in adult *Setaria* worms treated with the plant extracts at concentration of 1, 5, 10mg/ml and at two incubation periods are presented in Fig 1&2. The plant can be considered macrofilaricidal at an inhibition of >50%. This again provides quantitative picture of viability of the treated worms in comparison to heat killed and solvent control worms.

*Oldenlandia herbacea* caused 65.67 % inhibition in formazan formation at a minimum concentration 1 mg/dl at higher incubation period at 4 hours. At higher concentration and larger incubation period, significantly higher percentage inhibition 85.66% in formazan
formation was observed. *Oldenlandia herbacea* exhibited 65.67, 78.6 and 85.66 % of inhibition for the concentration of 1, 5 and 10 mg/ml respectively. *C.occidentalis*, *Oldenlandia herbacea* and *Sida acuta* exhibited > 50 % of formazan inhibition at 4 hours. *Clitoria ternatea* and *Euphorbia hirta* exhibited <50 % of inhibition at 4 hours. Except 8-10mg/ml, all other test concentration did not show significant increase in activity at an exposure period of 1 hr.

*Odenlandia herbacea* exhibited the maximum activity at the minimum concentration and exposure period of 4 hours, all other exhibited significant macrofilaricidal activity at higher concentration at higher incubation period.

**DISCUSSION**

Filariasis causes permanent and long term disability in developing countries. The complexities of the filarial disease and the absence of an effective adulticidal drug against the filarial parasite make elimination of the disease from the affected community more challenging. Natural products continue to represent important source for leads in new drug development of human use. Many clinically successful anti parasitic drugs are either natural products or developed from naturally occurring lead molecules. The macrofilaricidal potential of medicinal plants used in the traditional medicine for filariasis, anthelminitic and worm infestation activity has been assessed in the present study.

All the plant extracts were screened for macrofilaricidal activity against cattle filarial worm *S.digitata* invivo. Of these *Oldenlandia herbacea*, *Cassia occidentalis* and *Sida acuta* showed promising macrofilaricidal activity by worm motility assay and MTT reduction assay. MTT reduction a parameter has some advantages over motility suppression of motility does not always correlate with worm death, and there may be instances where worms may not be irreversibly damaged but merely paralysed and or able to recover. A similar observation in the case of *Sida acuta* and *Cassia occidentalis*, in the motility assay these two plants extracts need either a higher concentration or a longer duration of incubation in the treated medium to be macrofilaricidal. Possible observation may be due to its relatively slow actions macrofilaricides in the case of *Oldenlandia herbacea* plant. The methanolic extracts of the root *Plumbago indica/rosea* was screened for concentration of 1-10 mg/ml of the crude extracts caused complete immobilization of worms in 100 minutes of exposure.[6] Methonolic extracts of *Centratherum anthelminticum*, *Cedrus deodara*, *Sphaeranthus indicus* and *Ricinus communis* exhibited macrofilaricidal activity at concentration below 4 mg/ml at 100
minutes. Among these *Centrtherum anthelminticum*, was macrofilaricidal at 3mg/ml with motility suppression within 100 minutes. At lower concentration *Centrtherum anthelminticum*, showed no significant effect on motility whilst the activity of *C.deodara* titrated down to 2mg/ml, *S. Indicus* and *R.communis* to 1mg/ml with complete suppression of motility within 100 minutes.\(^4\)

Previous report states that *Cassia occidentalis* plant leaf extracts have antibacterial, antimalarial, mild analgesic, antipyretic,\(^6\) and anti-inflammatory activity.\(^7\) *Oldenlandia herbacea* is used in malaria, elephantiasis.\(^5\) Traditional medicines used for stimulation of the immune system, promoting the activity of the white blood cells and helps in clearing infection from the lymph glands. It is claimed to help in filariasis,\(^8\) hydrocele,\(^9\) swelling and oedema.\(^10\) *Sida acuta* is credited with anthelmintic, antiemetic and wound healing properties.\(^11\) It is also used to treat disorders of the blood, antiplasmodial, mosquitocidal activity, larvicidal, repellent activity.\(^12\) Its root is said to possess antimicrobial, antimalarial anti-oxidant, insecticidal activity.\(^13\) *Clitoria ternatea* used for worm infestation,\(^1\) anti-inflammatory, analgesic, antipyretic, immunomodulatory, immune inhibitory activity.\(^14\) *Euphorbia hirta* Linn Latex of this plant is used as application for warts\(^15\) and intestinal parasitosis.\(^16\)

The macrofilaricidal effect of the plants may be due to the damage caused to the DNA. 80% of the population depends on plant based medications. Traditional medicines are popular among the people and are being largely used to treat various ailments. Research in the use of traditional medicines for the treatment of filariasis is warranted in the present scenario as these drugs will provide a safe and efficacious drug against lymphatic filariasis.

**CONCLUSION**

This study revealed the macrofilaricidal properties of *Cassia occidentalis, Oldenlandia herbacea, Sida acuta*, and has also identified promising macrofilaricidal plants. The results obtained suggests that *Oldenlandia herbacea* possess significant macrofilaricidal activity on adult *S.digitata* invitro.

**REFERENCES**


