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Anti-microfilarial activity of methanolic extract of *Vitex negundo* and *Aegle marmelos* and their phytochemical analysis

K N Sahare[1], V Anandhraman[2], V G Meshram[3], S U Meshram[1], M V R Reddy[2], P M Tumane[1] and & K Goswami[2*]

[1] P G Department of Microbiology & Rajiv Gandhi Biotechnology Centre, RTM, Nagpur University, Nagpur 440 033, India
[2]Jamnalal Bajaj Tropical Disease Research Centre & Department of Biochemistry,
Mahatma Gandhi Institute of Medical Sciences, Sevagram 442 102, India
[3]Department of Biochemistry, Sindhu Mahavidyalaya, Nagpur 440 017, India

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In the present study, methanolic extracts of roots of *Vitex negundo* L. and extracts of leaves of *Vitex negundo* L., *Ricinus communis* L. and *Aegle marmelos* Corr. were explored for possible antifilarial effect against *Brugia malayi* microfilariae. It was observed that among the herbal extracts, root extract of *Vitex negundo* L. and leaves extract of *Aegle marmelos* Corr. at 100 ng/ml concentration showed complete loss of motility of microfilariae after 48 hr of incubation. Thin layer chromatography of the extracts revealed the presence of alkaloids, saponin and flavonoids in the roots of *Vitex negundo* L. and coumarin in the leaves of *Aegle marmelos* Corr.

Keywords: *Aegle marmelos,* Antifilarial activity, Microfilariae, Phytochemicals, *Ricinus communis, Vitex negundo*

Traditional therapeutics based on herbal medicinal principles is time tested and widely accepted across various cultural and socio-economic strata. The systematic screening of plant species for discovering new bioactive compounds are performed in many laboratories[1]. However, there is lack of precise guidelines to study the herbal compounds and till date a very meagre portion of this tremendous potential drug-repertoire has been scientifically screened[2]. Hence, there is a real need for scientific evidence based validation of these agents.

The tropical disease, human lymphatic filariasis, is still prevalent in central India. This disease, has been recognized by World Health Organization (WHO) as one of the ten diseases in its Tropical Disease Research (TDR) scheme highlighting the huge disease burden leading to 5.5 million DALYs. Consequently global programme was launched for elimination of filariasis (GPELF) (www.who.int/tdr/diseases). Currently most popularly used medicine, diethylcarbamazine (DEC), has been reported for lack of compliance due to prolonged use by mass drug administration strategy[3]. Hence there is dire demand for alternate options, which naturally relies on herbal remedies in terms of safety, efficacy and cultural acceptability.

Vitex negundo L. (Family: *Verbenaceae*) commonly known as Nirgundi, roots and *Ricinus communis* L. (Family: *Euphorbiaceae*) commonly known as castor oil plant or errand, leaves are used for elephantiasis[4, 5]. *Aegle marmelos* Corr. (Family: *Rutaceae*), known as bael, also used for treatment of filariasis[6]. The present study was undertaken to demonstrate the antifilarial effect of methanolic extract of these plants using *Brugia malayi* microfilariae *in vitro*. Preliminary phytochemical analysis and thin layer chromatography of two strongly active plant extracts namely roots of *Vitex negundo* L. and leaves of *Aegle marmelos* Corr. (Leaves) were also carried out to detect the major phytochemical groups present in these plants.

Materials and Methods

Plant material – Vitex negundo L. roots and leaves, *Ricinus communis* L. leaves and *Aegle marmelos* Corr. leaves, collected from the local areas of Sausar, Chhindwara (India), were identified by Dr. Alka Chaturvedi, P.G. Dept. of Botany, RTM, Nagpur University, Nagpur, India. (Vouchar specimen number 9022, 9025, and 9023, respectively).

Parasites — B. malayi life cycle was established and maintained in jirds (*Meriones unguiculatus*),

*Correspondent author
Phone: +91-7152-284741, Extn.262; Fax: +917152-284038
E-mail: goswamikln@rediffmail.com; goswamikln@gmail.com

mastomys (*Mastomys natalensis*) and mosquitoes (*Aedes aegypti*) by standard methods[7, 8]. 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 (containing 20 µg/ml, gentamycin; 100 µg/ml, penicillin; 100 µg/ml, streptomycin; from Himedia Laboratories Pvt. Ltd, Mumbai) plated on sterile plastic petridishes and incubated at 37°C for 1 hr to remove jirds peritoneal exudate cells. The mf were collected from petri dishes, washed with RPMI 1640 medium and used for *in vitro* maintenance[9].

Preparation of plant extract — Plants materials were dried in oven at 37°C and powdered. Roots of *V. negundo* L. were extracted in methanol (99.99%) by reflux apparatus at 55°-65°C for 9-10 hr with 5.2% yield[10], while, leaves of *V. negundo* L and *R. communis* L. were extracted in methanol (70%) by cold maceration at room temperature for 24 hr (yields 4.2 and 4.7 %, respectively). Leaves of *A. marmelos* Corr. were extracted similarly in methanol (99.99%) with 3.7% of yield[11]. All the extracts were stored in refrigerator (4°C) for future use.

In vitro screening of plant extract for anti-microfilarial activity against B. malayi — Dilutions of crude extract of medicinal plants were made in methanol and finally diluted to 3% with RPMI 1640 medium. The diluted extract (900 µl) was poured in 24 wells of sterile culture plates (Nunc, Denmark) maintaining concentration of the extracts at 100 ng ml^{-1}. Controls were also kept without the test solution, but with methanol (3%) and RPMI medium (900 µl). Approximately 100 microfilariae in 100 µl of RPMI 1640 medium were introduced into each well. Both test and control wells were taken in duplicates. The plates were incubated at 37°C for 24 and 48 hr in CO_2 incubator (5%CO_2). After exposure to extracts followed by exposure to extract free fresh medium for 1 hr, the number of live and dead mf in each well was counted[12]. Each experiment was repeated thrice. The entire procedure was carried out under aseptic conditions.

Statistical analysis — Data was subjected to statistical analysis using Student's *t*-test to compare the mean percentage of reduction in motility with respective controls.

Preliminary phytochemical analysis — Phyto-chemical screening of methanolic extracts of *V. negundo* L. roots and *A. marmelos* Corr. leaves were undertaken using standard methods to check for the presence of alkaloids, phenols, steroids, terpenoids, saponins, coumarins and flavonoids.[13]

Thin layer chromatography (TLC) — TLC of methanolic extracts of roots and leaves of test plants was carried out using standard methods (Phenol[14] Saponin, Flavonoids[15], Steroids[10], Coumarins[15], Alkaloids[10] and Terpenoids[16]).

Results and Discussion

In the present study, four methanolic extracts derived from different plants namely *Vitex negundo* L. (Roots), *Vitex negundo* L. (Leaves), *Ricinus communis* L. (Leaves) and *Aegle marmelos* Corr. (Leaves) were explored for possible antifilarial effect on *Brugia malayi* microfilariae *in vitro* and percentage reduction in terms of motility after 48 hr were summarized in Table 1. Among the four extracts tested in culture medium, methanolic extracts of roots of *Vitex negundo* L. and of *Aegle marmelos* Corr. showed complete loss of microfilarial motility after 48 hr exposure which was highly significant compared to respective controls. Hence, significant level of anti-microfilarial effect observed with roots of *Vitex negundo* L. and leaves of *Aegle marmelos* Corr. as compared to control supports their medicinal use. However, methanolic extracts of leaves of *Vitex negundo* L. and *Ricinus communis* L. were not found to achieve similar efficacy like the other two, possibly indicating their less direct effect on the parasite.

In an effort to find out the phytochemical components of the two plant extracts with higher

Table 1 — *In vitro* screening results of medicinal plant extracts (at 100ng/ml conc. of each extract) against microfilariae of *Brugia malayi*

[Values are mean ± SE of 3 observations]

Plant extracts	Reduction in Microfilarial motility (%)	
	After 24 hr	After 48 hr
[1]*Vitex negundo* L. Roots	86.82 ± 1.07*	#
[2]*Vitex negundo* L. Leaves	15.23 ± 1.29*	44.66 ± 2.540*
[2]*Ricinus communis* L. Leaves	22.23 ± 1.51*	25.9 ± 1.89*
[1]*Aegle marmelos* Corr. Leaves	35.11 ± 1.50*	#
Control 1	6.58 ± 0.75	14.16 ± 0.549
Control 2	10.23 ± 2.36	14.52 ± 1.173

*Significant at the level of $P< 0.05$ when compared with respective control (Control 1- RPMI + methanol (70%); Control 2- RPMI+ methanol (99.9%)

Complete loss of motility was recorded

[1] Roots and leaves extracts in methanol (99.9%)[2], Leaves extracts in methanol (70%)

antifilarial efficiency, preliminary chemical and chromatographic (TLC) analysis was carried out. The result revealed that roots of *Vitex negundo* L. had alkaloids, saponin, and flavonoids and the leaves of *Aegle marmelos* Corr. contained coumarins (Fig. 1).

Vitex plants reportedly contain vitexicarpin as active principle; which has flavonoid analogous structure with cytotoxic effect[17]. Apart from well documented antioxidant role, flavonoids behave as pro-oxidants[18]. Apoptosis may be induced by oxidative stress[19] and more interestingly peroxynitrite derivative generated from the interaction between nitric oxide and reactive oxygen intermediates has been reported to augment flavonoid associated apoptotic impact[20]. Recently, nitric oxide has been implicated in the macrophage mediated sequestration of filarial parasites *in vivo*[21]. Hence, it appears that flavonoids detected in this plant extract might be responsible for antifilarial effect. *Vitex* root extract was also found to contain saponins. Saponins by nature are detergents with steroidal structure (www.http://en.wikipedia.org/wiki/Saponin) which might be expected to affect bio-membranes. Certain saponins are found to be apoptotic also[22]. Saponins along with other flavonoids, polyphenols, tannins and coumarins were shown to be responsible for different antimicrobial effects of *Cylicodyscus gabunensis* plant extract[23], which is traditionally known for medicinal use against headache, filariasis, rheumatism and gastrointestinal disorders[24]. In this study, we detected alkaloid from *Vitex* roots the exact chemical nature of which was not further resolved. However,

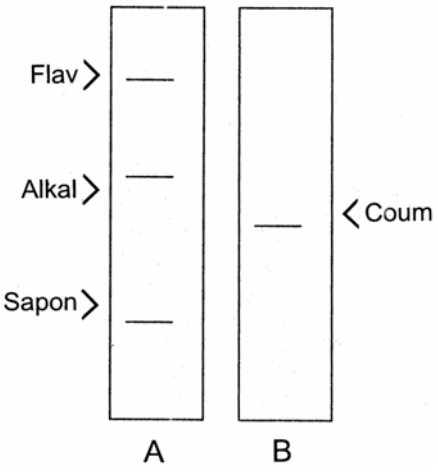

Fig: 1— Diagrammatic representation of the results obtained in TLC for the two active plants tested. [Flav-Flavonoids; Alka-Alkaloids; Sapon-Saponin; and Coum-Coumarin; *A- V. negundo; B- A. marmelos*]

certain synthetic alkaloids have been reported to have antifilarial activity *in vivo*[25], which supports the view that further work with alkaloid derivatives may be rewarding.

Other plant extract that showed significant antifilarial activity *Aegle marmelos* Corr. (Leaves), belongs to Rutaceae group which is reported to contain various flavones[26]. However, we did not find flavonoids in leaves extract of *A. marmelos*. Rather, as found earlier with certain other members of this group[27] coumarins were detected. Coumarins are chemically benzopyrone derivatives with myriad pharmacological properties and some of them are proved in reducing lymphoedema associated with elephantiasis[28]. Of late, these compounds have been shown to be effective against *Brugia malayi* both *in vivo* as well as *in vitro* as topoisomerase II inhibitors[29].

The present work confirme the antifilarial effect *of V. negundo* and *A. marmelos* and initial phytochemical analysis provided important mechanistic clue for the activity. Hence, future work with such compounds may be undertaken for antifilarial activity.

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