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Antifilarial potential of the root extracts of *Mirabilis jalapa* Linn. (Nyctaginaceae) on cattle filarial parasite *Setaria cervi*

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SUMMARY

Effect of aqueous and alcoholic extracts of the roots of *Mirabilis jalapa* Linn. Four Oclock plant, on the spontaneous movements of both the whole worm and the nerve-muscle (n.m.) preparation of *Setaria cervi* and on the survival of microfilariae *in vitro* was studied. Alcoholic extract caused the inhibition of spontaneous movements of the whole worm and the n.m. preparation of *S. cervi*, whereas aqueous extract caused inhibition of spontaneous movements of the n.m. preparation. The initial stimulatory effect was not observed by aqueous and alcoholic extracts on n.m. preparation while effect of alcoholic extract on the whole worm was characterized by an increase in the amplitude of contractions followed by reversible paralysis. The concentrations required to inhibit the movements of the whole worm and n.m. preparation for alcoholic extract of root were 270 μg/mL and 40 μg/mL, respectively whereas an aqueous extract caused inhibition of n.m. preparation at 30 μg/mL suggesting a cuticular permeability barrier. Alcoholic extract of the roots of *M. jalapa* caused concentration related effect on the survival of microfilariae of *S. cervi*. The LC_{50} and LC_{90} for alcoholic extract as observed after 6 hrs. were found to be 10 ng/mL and 18 ng/mL., respectively.

Keywords: *Mirabilis jalapa* Linn., Nyctaginaceae, *Setaria cervi* antifilarial activity, Microfilaricidal

INTRODUCTION

Tubers of *Mirabilis jalapa* Linn. (Nyctaginaceae) were once erroneously thought to be the source of Jalap (Exogonium purga) and are mildly purgative and used as a substitute or adulterant of true Jalap. Tuber is used as a poultice on carbuncles. Rubbed with water is applied on contusions (The Wealth of India, 1962; Chopra *et al.*, 1956). Phytochemical studies of *M. jalapa* revealed the presence of several terpenoidal compounds including transphytol, Me-3-oxoures-12-ene-28 oate, β-sitosterol, stigmasterol, brassicasterol, β-sitosterol acetate, oleanolic acid, ursolic acid, (Siddiqui *et al.*, 1994), α-amyrin, α-amyrin acetate (Begum *et al.*, 1994), β-sitosterol-D-glucoside, and β-amyrin-3-O-α-L-rhamnosyl-O-β-D-glucoside (Saxena and Gupta, 1986), amino acids (Rastogi *et al.*, 1982), carbohydrates (Ray *et al.*, 1988), C_{23}-C_{35} straight chain alkanes, 12-tricosanone, n-hexacosanol, tetracosonic acid, tartaric acid, citric acid (Behari *et al.*, 1976), 8-hydroxyoctadeca-cis-11,14-dienoic acid (Ahmad *et al.*, 1984), 2-O-methylabron-isoflavone, 9-O-methyl-4-hydroxy-boeravinone B, 6-methoxy boeravinone (Yang *et al.*, 2001), betalain (Doepp and Musso, 1974), miraxanthin I, II, III, IV, indicaxanthin, vulgaxanthin I (Piatteli *et al.*, 1965), and antimicrobial peptides (De Bolle *et al.*, 1996; Cammue *et al.*, 1992).

No report appears in the literature suggesting its role as an anthelmentic. During routine screening, the ability of the roots extract to inhibit the spontaneous mobility of filarial parasite *Setaria cervi* generated interest and it was thought worthwhile to explore anthelmentic potential of the two extracts (alcoholic and aqueous). The filarid worm, *Setaria cervi* (Rudolphi, 1819) is a cosmipolitan nematode parasite inhabiting the paritonial cavity of buffaloes (*Bubalus bubalis* Linn.).

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Sitariasis is a very common in India showing incidence as high as 85 per cent (Wajihullah, 2001). The parasite is also known to cause "Cerebrospinal nematodiasis" in the parasitised hosts (Pachauri, 1972). *S. cervi*, resembles closely to human filarial worms in its response to drugs and can therefore be used for the screening of potential antifilarial agents (Singhal *et al.*, 1969; 1972; 1973). *S. cervi* exhibits vigorous rhythmical movements, which can be recorded on a kymograph by suspending the worm in an isolated organ bath. Also, the nerve-muscle preparation of the worm exhibits similar movements (Singhal *et al.*, 1977). The present study was designed to observe the effect of the aqueous and alcoholic extracts of the roots *M. jalapa* on the spontaneous movements of the whole worm, nerve-muscle (n.m.) preparation and on the survival of microfilariae of *S. cervi in vitro*.

MATERIALS AND METHODS

Plant Materials

The roots of *M. jalapa* were collected from the Survey of Medicinal Plant Unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India. The plant was identified by Dr. Athar Ali Khan, Department of Botany, A.M.U., Aligarh, where the voucher specimen (# 1140) has been deposited.

Collection of *Setaria cervi*

Motile adult *S. cervi* (Nematoda: Filariodea) of average length 6.0±1.0 cm and of average weight 35±6.0 mg were obtained from the freshly slaughtered cattle (*B. bubalis* Linn.) and brought to the laboratory in a vacuum flask containing modified Ringer's solution (NaCl 9 g, KCl 0.42 g, $NaHCO_3$ 0.5 g, $CaCl_2$ 0.24 g, glucose 0.25 g in 1L distilled water) at 37°C (Singhal *et al.*, 1973). The time period between the removal of the worms from the host to the laboratory was less than 3 hours. In the laboratory, the worms were repeatedly washed with the same solution to free them of any extraneous material.

Whole worm preparation

Adult *S. cervi* was suspended in an isolated organ bath of 20 mL capacity, in modified Ringer's Solution at 37°C. *S. cervi* exhibits vigorous rhythmical movements, which can be recorded on a kymograph by suspending the worm in an isolated organ bath. Spontaneous movements of the worm were recorded on a slow moving drum (Singhal *et al.*, 1975), aeration was not required as it did not improve the motility of the worm. About 15 minutes were allowed for the movements of the worm to stabilize before eliciting the response to the drug. The drug was added in increasing concentrations to the bathing fluid and allowed to remain in contact for 15 minutes, if there was no response it was considered inactive. A fresh worm was used to test each concentration of the extract. This precaution was taken to avoid a cumulative response of the residual drug in the worm.

Nerve-muscle (n.m.) preparation

A worm was placed in a petridish containing modified Ringer's solution. Two dissecting needles were inserted at one end of the worm and the cuticle was split longitudinally in one stroke. The anterior 1 cm of the worm was cut off to eliminate the influence of the nerve ring and the cephalic ganglia. The remaining part was tied at both ends and suspended in the isolated organ bath containing modified Ringer's Solution at 37°C. The nerve-muscle preparation showed rhythmical spontaneous movements, which were recorded on the slow moving kymograph.

Collection of microfilariae

The uterus of a female *S. cervi* was cut at its junction with the vagina and just below the bifurcation and removed from the worm. The uterus was teased with a needle in the solution and microfilariae were freed. The microfilariae were suspended in human serum: Ringer mixture, the count was adjusted to 100/mL, and 0.5 mL aliquots of the microfilariae suspension were placed in sterilized screw capped bottles containing aqueous or alcoholic extracts of *M. jalapa* in an equal human serum: Ringer mixture (v/v). *M. jalapa* extract was added in doubly increasing concentrations of 5 ng/mL. The bottles were kept in an incubator at 37°C and examined under a microscope after 6 hours, to count the living and dead microfilariae. The LC_{50} and LC_{90} were calculated from a concentration/death graph. In a preliminary set of experiments it was ascertained that the concentration

of alcohol/water in the suspending medium did not influence the survival/motility of the microfilariae.

In a preliminary experiment, the aqueous and alcoholic extracts of the roots of *M. jalapa* were added to microfilariae in concentrations of 5, 10, 15, 20 and 25 ng/mL to determine the limits of activity within 6 hours at 37°C. Within these limits, six concentrations were selected to observe the survival of microfilariae. The effect of each dose was observed 5 times. The mean of the values was plotted on a graph. All analyses were done using the SPSS 7.5 for windows. Experimental values were expressed as Mean ± SEM (Standard Error of Mean). Statistical significance was considered to be indicated by a p value of less than 0.05 in all cases.

Preparation of Extract

Dried and powdered roots of *M. jalapa* were extracted with ethanol and water, separately. The crude ethanol and aqueous extracts were dried and dissolved in 95% ethanol and distilled water before use. The addition of 0.2 to 0.5 mL vehicle (95% ethanol or distilled water) to the organ bath containing 20 mL Ringer's solution had no effect on worm motility.

RESULTS

Effect of alcoholic extract of the roots of *M. jalapa* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi*

A typical response of alcoholic extract of the roots of *M. jalapa* on the spontaneous movements of the whole worm of *S. cervi* is shown in Fig. 1. Addition of extract in a concentration of 50 μg/mL to the bath fluid caused an increase in the amplitude of contractions. The rate and tone of contractions were however not affected. The effect was evident immediately after the addition of the drug. The increase in amplitude continued for nearly two hours after that the rate and amplitude started declining and continued to do so for more than 6 hours. With a higher concentration of 270 μg/mL the stimulant effect of the drug characterized by increase in amplitude and small decrease in tone of contractions was followed by cessation of worm movements early as compared to smaller concentration of 50 μg/mL. The worm continued to remain paralysed for more than 6 hr. However, with repeated changes of the bathing fluid, the movements of the worm were slowly restored to normal. This indicates that paralysis caused by higher concentration of alcoholic extract of roots was reversible in nature (Fig. 2).

On n.m. preparation the alcoholic extract of the roots of *M. jalapa* produced decrease in spontaneous movements characterized by decrease in amplitude, rate and tone of the contractions. This effect continued till the paralysis of the n.m. preparation. The initial stimulant effect was not observed. It took about 45 minutes for a concentration of 40 μg/mL to completely paralyse n.m. preparation (Fig. 3). However, with repeated changes of the bathing fluid (w), the movements of the n.m. preparation were restored to normal. This indicates that

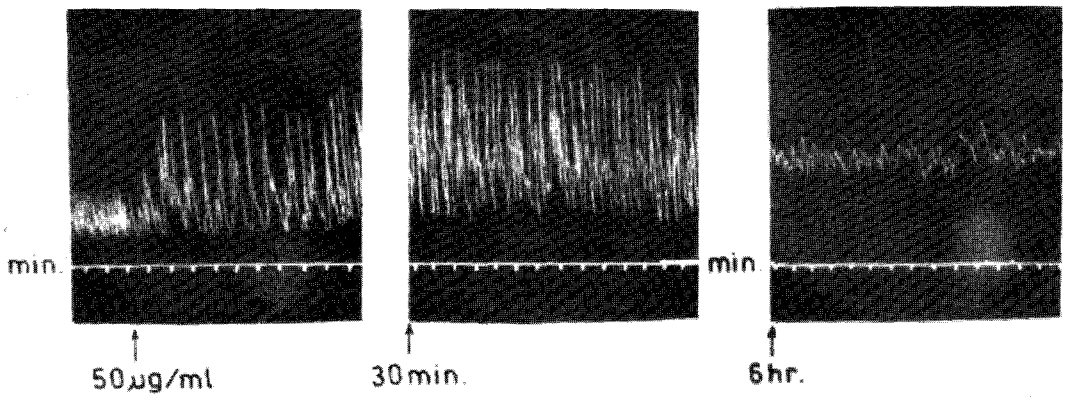

Fig. 1. The stimulatory effect of 50 μg/mL alcoholic extract of the roots of *M. jalapa* on the spontaneous movements of the whole worm preparation of *S. cervi*. The addition of extract to the bath fluid caused an increase in the amplitude of contractions. The rate and tone of contractions were however not affected.

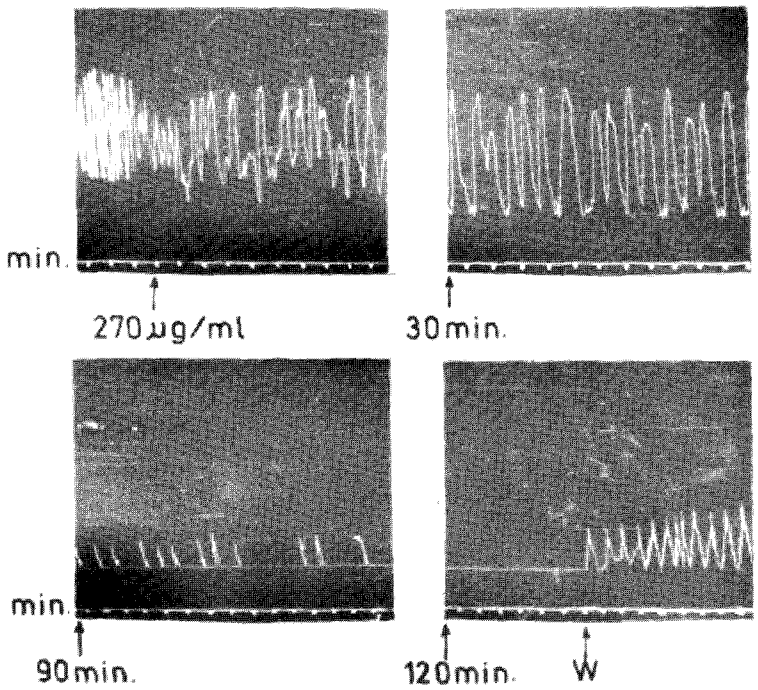

Fig. 2. The reversible paralysis by 270 µg/mL alcoholic extract of the roots of *M. jalapa* on the spontaneous movements of the whole worm preparation of *S. cervi*. The stimulant effect of the drug characterized by increase in amplitude and small decrease in tone of contractions was followed by cessation of worm movements early as compared to smaller concentration of 50 µg/mL.

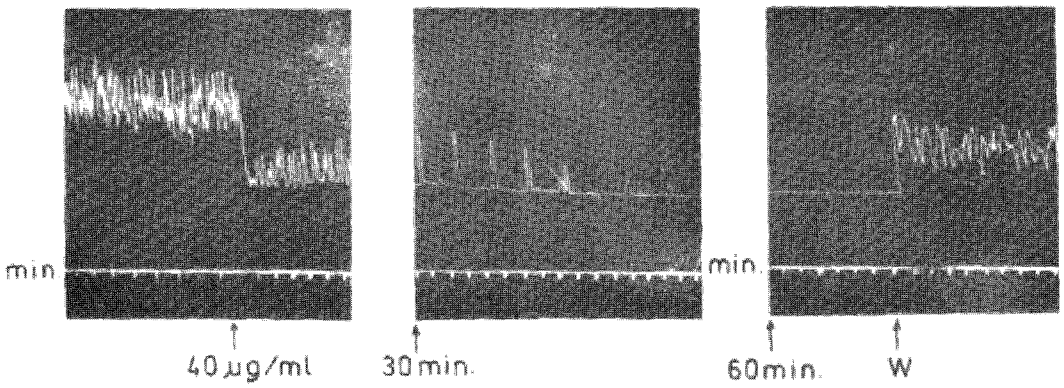

Fig. 3. The reversible paralysis by 40 µg/mL alcoholic extract of the roots of *M. jalapa* on the spontaneous movements of n.m. preparation of *S. cervi*. The initial stimulant effect was not observed. The alcoholic extract produced decrease in amplitude, rate and tone of contractions.

paralysis caused was reversible in nature.

Effect of aqueous extract of the roots of *M. jalapa* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi*

No significant response was observed on the whole worm by aqueous extracts of the roots of *M. jalapa* at any concentration. A typical response of aqueous extract of the roots of *M. jalapa* on the spontaneous movements of n.m. preparation was observed which has been shown in Fig. 4. The response of aqueous extract on n.m. preparation is quite interesting. The aqueous extract produced decrease in amplitude, rate and tone of contractions immediately following the addition of drug to the bath fluid. It took about 25 minutes for a concentration of 30 µg/mL to completely paralyse n.m. preparation. The movements were however

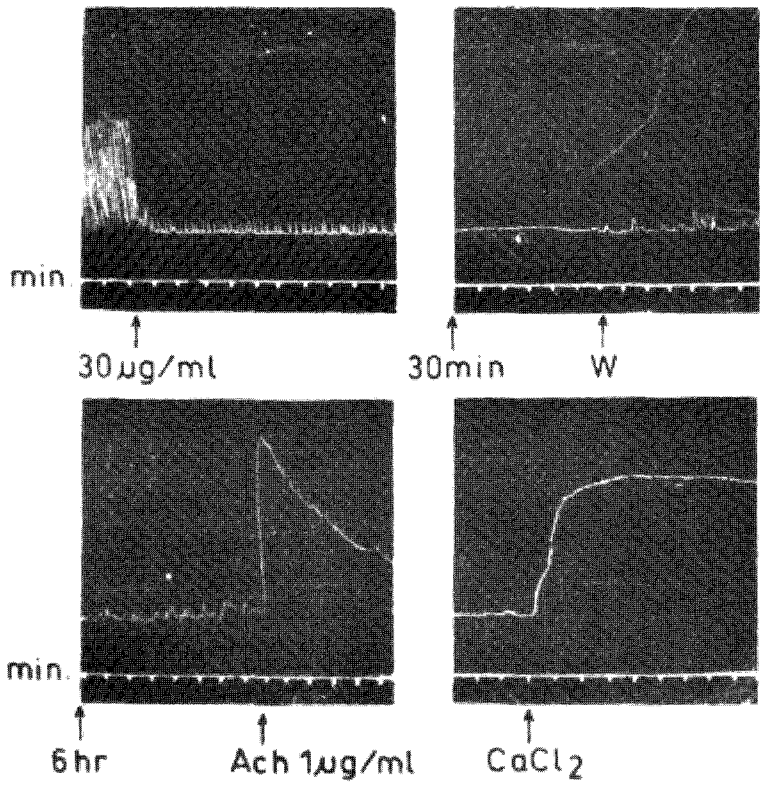

Fig. 4. The partial irreversible paralysis by 30 μg/mL aqueous extract of the roots of *M. jalapa* on the spontaneous movements of n.m. preparation of *S. cervi*. The aqueous extract produced decrease in amplitude, rate and tone of contractions immediately following the addition of drug to the bath fluid. Addition of acetylcholine (Ach.) an excitatory neurotransmitter to the bath fluid, and $CaCl_2$ could elicit the response.

not restored despite repeated changes of the bathing fluid (w). This indicates that the paralysis caused was irreversible. There was no indication of restoration of movements even after 6 hour. Addition of acetylcholine (Ach) an excitatory neurotransmitter to the bath fluid, and $CaCl_2$ could elicit the response (Fig. 4).

Effect of alcoholic and aqueous extracts of the roots of *M. jalapa* on the survival of microfilariae of *S. cervi in vitro*

Aqueous extract of *M. jalapa* failed to effect the survival of microfilariae to any significant extent up to a concentration of 500 ng/mL. Alcoholic extract, however showed concentration relation inhibition of motility and death of microfilariae. The survival of microfilariae of *S. cervi* was significantly reduced in the presence of alcoholic extract of roots of *M. jalapa* with a concentration of 10 ng/mL (LC_{50}) of the extract, the survival of microfilariae was significantly reduced after remaining in contact for 120 minutes ($p<0.005$) and continued to remain so till the observation period of 6 hrs. Similarly with a higher concentration of 18 ng/mL (LC_{90}) the survival of microfilariae was reduced but a little earlier and significant reduction in survival was observed at 90 min and only 11 percent microfilariae survived till 6 hrs as compared to 86% in the control group (Table 1). The LC_{50} and LC_{90} calculated from mean of survival at 6 hrs were 10 and 18 ng/mL, respectively, which is shown in Fig. 5.

DISCUSSION

It is interesting to note that the effect of the alcoholic extract of the roots of *M. jalapa* on both the whole worm and the n.m. preparation was reversible in nature, whereas the aqueous extract produced irreversible paralysis of the *Setaria* n.m. preparation. Aqueous extract did not show any significant effect on the whole worm as well as on

Table 1. Effect of alcoholic extract of the roots of *M. jalapa* on the survival of icrofilariae of *S.cervi in vitro* [Mean ± SEM (p values)]

	Survival (%)		
Times (minutes)	Controlled	LC_{50} (10 ng/ml)	LC_{90} (18 ng/ml)
0	100.00	100.00	100.00
30	98.00±0.567	96.00±0.707	96.00±0.948
60	97.00±0.474	95.00±0.707	90.00±1.732
90	96.00±0.707	91.00±1.140	80.00[a]±2.333
120	95.00±0.836	88.00[a]±1.378	75.00[a]±2.848
150	93.00±1.224	87.00[a]±1.224	70.00[a]±2.185
180	92.00±0.707	75.00[a]±2.213	60.00[b]±3.144
210	92.00±1.00	70.00[a]±1.140	55.00[b]±2.924
240	91.00±1.581	70.00[a]±2.024	45.00[b]±2.284
270	90.00±1.048	65.00[b]±1.516	30.00[b]±3.415
300	89.00±1.414	60.00[b]±1.643	25.00[b]±2.867
330	88.00±1.140	56.00[b]±1.760	14.00[b]±1.855
360	86.00±1.140	53.00[b]±1.843	11.00[b]±1.201

Experiments are indicated in the parenthesis. a=p <0.005, b=p <0.001 compared to controlled.

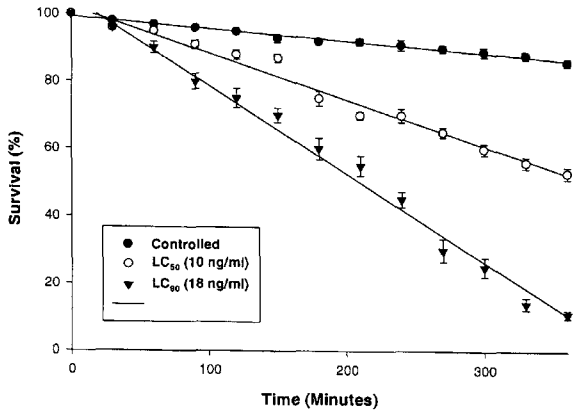

Fig. 5. Effect of alcoholic extract of the roots of *M. jalapa* on the survival of microfilariae of *S. cervi in vitro*. All experiments were performed in pentaplate and bars representing standard errors of mean are shown.

microfilariae of *S. cervi*. This clearly indicates that both extracts are having different effecting substances. Alcoholic extract produced stimulant effect on the whole worm followed by reversible paralysis. On the other hand no stimulation was observed on the n.m. preparation. It appears that initial stimulation observed on the whole worm is due to the irritant effect of the alcoholic extract on the cuticle of the worm. With the removal of the cuticular barrier the stimulant effect was not visible. Aqueous extract of roots on the other hand produced no initial stimulation followed by irreversible paralysis on n.m. preparation. However, the addition of Ach. and $CaCl_2$ during paralysant phase caused short lasting stimulant effects seen only as a spike. On the other hand it was inactive against whole worm. This is clearly suggesting cuticular permeability barrier. The effect therefore could be due to partial blockade of acetylcholine (Ach.) receptors but not due to blockade of calcium channels. The other possibility is that the response of *Setaria* to the root extracts of *M. jalapa* observed in the present study resembles diethylcarbamazine (DEC), a known antifilarial agent in that low doses cause stimulation characterized by increase in amplitude followed by paralysis. Diethylcarbamazine has also been shown to decrease the glucose uptake by the adult worm of *S. cervi* suspended in modified Ringer's solution (Singhal *et al.*, 1978). Further DEC produces reversible dose dependent deplorization of the membrane potential of another nematode *Ascaris Suum* by antagonizing voltage sensitive K^+ conductance in the muscle (Martin *et al.*,1982). The effect of DEC on filarial parasite is said to be obscure (Maizles *et al.*, 1992). The human filarial parasite lives in tissues eg. *Wucharia bancrofti* and *Brugia malayi* cause elephantiasis by blocking the lymphatics. It cannot be predicted whether DEC ever reaches the adult worm, but there is presumptive evidence that DEC sterilizes the adult worm and even cause paralysis and may

cause death. However, it is the microfilariae, which live in circulation are exposed to the drug. DEC does not kill the microfilariae in circulation but sensitizes the microfilariae to the action of fixed macrophages, which kill them (Hawking *et al.*, 1948), which could be the possibility in this case. *M. jalapa* root extracts may provide a chemical lead for the synthesis of new derivatives which might prove to be potential antifilarial agents.

On the microfilariae of the *S. cervi*, alcoholic extract of the roots of *M. jalapa* reduced the survival time in a concentration related manner. The LC_{50} and LC_{90} being 10 and 18 ng/mL, respectively. If this concentration could be presented to the microfilariae *in vivo*, the extract could be a useful tool for the treatment of filariasis.

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