Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (4):1985-1987
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Lavacidal activity of *Citrus paradisi Macf.* and *Murraya koenigii spheng.* (Rutaceae) extracts on microfilaria larva of *Simulium yahense*

[1]Ebigwai J.K., [1]Edu E.A., [2]Obi-iyeke G.E., [3]NTA I.A.

[1]Department of Botany, University of Calabar, Nigeria
[2]Department of Botany, Delta state university, Abraka
[3]Department of Zoology, University of Calabar, Nigeria

ABSTRACT

Laboratory bioassay of methanol extracts of Murraya koenigii, Citrus paradisi and their combinations were evaluated against the third infectious stage of Similium yahense .The in vitro test with concentrations ranging from 0.5%-5.0% revealed that the extract of the plants and their combination posses lavacidal activities as they could induce significant mortalities of the microfilaria on a dose dependent response. The LC50 values estimated for M.koenigii C. paradisi and their combinations are 1.96, 1.85 and 1.67 respectively. The extract obtained from their combination was found to be more effective while the extract obtained from M.koenigii was shown to be the least effective.

INTRODUCTION

Natural products and secondary metabolites formed by living systems, notably from plant origin, have shown great Potential in treating human diseases such as cancer, coronary heart diseases, diabetes, malaria and infectious diseases. At the moment, attention is being paid to the application of these natural plant products through the use of lavicides and ovicides as ideal control measures (Szirmai, 2005). Research works on the control of onchocerciasis and other related infectious diseases using synthetic products (ivermectin and diethyl carbazamine) abound in literatures. Nonetheless, literatures on the use of these natural products on the control and management of onchocerciasis are scanty at best and nonexistent at worst. It was estimated that West Africa share about 30% of global cases of onchocerciasis with 96% of reported cases globally occurring in Africa (Szirmai, 2005). It is against this back drop that this study aims at evaluating the larvacidal properties of *M koenigii* and *C.paradisi* against the infectious larva stage of *Simulium yahense* . In the present investigation, specific action and joint action of the methanol extracts (ME) of these two plant seeds were evaluated against the third infectious stage (microfilaria) of *Simulium yahense*.

MATERIALS AND METHODS

Sample Collection

Two medicinal plants namely *Murraya koenigii* (seed) and *Citrus paradisi* (seed) were collected from Ugbodu in Aniocha north local government area of Delta state, Nigeria. Seven herbalists from randomly selected communities in the area were interviewed on their methods of treatment of onchocerciasis which is prevalent in the area. After the

interview six plants were mentioned as anti-onchocerciasis herbs and two seeds were mentioned as possessing anti lavacidal properties. All of them were unanimous in their use of the herbs in combination for enhanced efficacy and their mode of preparation of the herbs (boiling or maceration in alcohol). The seeds of the plants were obtained through the help of two herbalists from the village forest. In addition, their respective leaves and stems were collected for botanical identification. The identification and authentication was done at the herbarium unit of the department of Botany, university of Calabar, Nigeria, where a voucher specimen was deposited.

Extraction

The freshly collected seed of *Murraya koenigii* spreng. (L.) Roxb.(Rutaceae) and *Citrus paradisi* L.(Rutaceae) and their combinations were sun-dried at ambient temperature (27 ± 0.5^0C) and powdered in a domestic grinder and stored in a refrigerator for further use. From the stock 10 gm of powder was used for the extract by the cold method. The powder was dissolved in 100 ml of methanol/DW in an air tight separating funnel for about 7 days. After this period the final volume was measured and the concentration assumed as 100%. From this stock, different concentrations were prepared in water medium and used for the LC50 test.

Larvae collection and Bioassay

The larvae were collected from Nigerian Institute of Medical Research, Yaba. Larvicidal bioassay was performed on the infectious third stage microfilaria. Twenty-five larvae were released into 500ml glass beakers containing 250ml distilled water. The larvae were provided a mixture of Dog biscuit, yeast powder and algae in a ratio 3:1:1 ratio as nutrients and supplemented with treatments by taking 0.5% (500 iL), 1.0% (1000 iL), 1.5% (1500 iL), 2.0% (2000 iL), 2.5% (2500 iL), 3.0% (3000 iL),3.5% (3500 iL), 4.0% (4000 iL), 4.5% (4500 iL) and 5.0% (5000 iL) from both extracts and their combination. The experiment was carried out at 27 ± 0.5^0C and three replicates of each concentration were run under the same microclimatic conditions along with untreated control. Mortality of the larvae was monitored at 24-48 hours post treatment. Bioassay test showing more than 15% control mortality was discarded and repeated . However, when control mortality ranged from 5-15%, the corrected mortality was corrected using Schneider-Orelli's formula, Puntener, (1981). The data obtained were subjected to probit analysis in order to estimate the LC50 and the heterogeneity values, (Finney 1952).

RESULTS AND DISCUSSION

The results of the study indicated that in vitro methanol extracts of *M. koenigii* and *C.paradisi* and their combination showed a very good activity against the larvae of *Simulium yahense*, when given at graded concentrations of 0.5%,1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5% and 5.0% respectively.(Table 1). Comparative analysis of the test indicated dose dependent responses, that is, as concentration increases, larval mortality increases. A high larval mortality was observed between 3.5%-4.5% corresponding to 3500 to 4500 iL concentration. At concentrations above the upper limit of the optimum range, percentage larval mortality exhibited a marginal decrease.

Conc.%	Probit values after Corrected Larval mortality for *C. paradisi extract.*(±)	Probit values after Corrected Larval mortality for their combination.(±)	Probit values after Corrected Larval mortality for *M. koenigii extract.* (±)	Mean larval mortality in minutes for *C.paradisi*	Mean larval mortality in minutes for their combination	Mean larval mortality in minutes for *M. koenigii*
control	-	-	-	245±0.75	254±0.75	230±0.75
0.5	3.36±1.67	-	3.66±1.33	175±0.75	142±0.75	198±0.75
1.0	3.36±1.67	3.66±1.33	3.92±1.67	162±0.75	138±0.75	184±0.75
1.5	3.66±1.33	3.66±1.33	4.26±1.55	159±0.75	128±0.75	172±0.75
2.0	4.08±1.55	3.92±1.67	4.26±1.55	152±0.75	120±0.75	165±0.75
2.5	4.39±1.67	4.26±1.55	4.39±1.67	145±0.75	102±0.75	150±0.75
3.0	4.64±1.37	4.39±1.67	4.77±0.69	133±0.75	91±0.75	124±0.75
3.5	5.00±0	4.77±0.69	5.13±1.55	123±0.75	75±0.75	105±0.75
4.0	5.28±1.67	5.00±0	5.74±0.78	105±0.75	62±0.75	100±0.75
4.5	5.13±1.55	4.64±1.37	6.08±0.33	112±0.75	60±0.75	98±0.75
5.0	4.75±0.77	4.64±1.37	5.47±0.67	119±0.75	61±0.75	98±0.75

±; Standard error

Similarly the time taken to observe mean larval mortality in minutes was lower at higher concentrations and vice versa. Comparatively, the concentrations and time needed to completely inhibit 1% of the microfilaria larvae varies among the tests. For instance, 5.0% concentration of the *Murraya* extract recorded the least time (1.45 mins) needed

to completely inhibit 1% of the infectious larva, while concentration of 4.0% recorded the least time required to effect same percentage decrease in the extract of *C.paradisi* species and the combined extract (2.57 and 2.39 respectively). This difference in time between *Murraya* extract on one hand and the other two extracts on the other hand was calculated to be statistically significant ($p<0.025$) at the 0.05 confidence limit.

The data obtained was subjected to probit analysis in order to estimate the LC50 values. In each of the extract and their combination, it was observed that the control mortality yielded responses of 12% larvae inhibition, thereby requiring mortality correction using Schneider-Orelli's formula. The LC50 estimated for *C.paradisi*, *M.koenigii* and their combination were 1.85%,(1850iL) 1.67% (1650iL)and 1.96%(1960iL) respectively. Heterogeneity values ($p<0.05$) calculated for *C. paradisi*, *M.koenigii* and their combination were 0.021, 0.024 and 0.001respectively. From the result, while, 1.67% of the combination of *C. paradisi* seed and *M.koenigii* seed was needed to kill 50% of the microfilaria larva, 1.85% of *C. paradisi* and 1.96% of *M.koenigii* was required to kill off same percentage(50%) of the microfilaria larva of *Simulium yahense*.

Table 2: Dosage response of *C. paradisi, Murraya koenigii* and their combination against the microfilaria larvae of *Simulium yahense*

Extracts	LC50 values (%)	Heterogeneity p (n)
Citrus paradisi	1.85	0.021(3)
Murraya koenigii	1.96	0.024(3)
Combination	1.67	0.001(3)

LC50; lethal concentration needed to kill 50% of the population exposed: p< 0.05 for heterogeneity; n: no of replicates

Earlier studies on the control and management of onchocerciasis was based on the use of ivermectin,(avermectin) an anti parasitic agent isolated from the fermentation products of *Streptomyces avermitilis (*Craven *et al.*, 2002*).*

REFERENCES

[1] Craven, J., Bjorn, H. and Hennessy, D.R.(**2002**). *J. Vet Pharmacol Ther*.;25(3):227-232.
[2] Finney, D. J., Ed. (**1952**). Probit Analysis. Cambridge University Press, Cambridge, England,
[3] Püntener W.(**1981**). Manual for field trials in plant protection second edition. Agricultural Division, Ciba-Geigy Limited, Basle.
[4] Szirma, A. (**2005**). The dynamics of socio-economic development: an introduction. Cambridge university press, Cambridge. 233p.