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In vivo evaluation of the essential oil extract of six plant species and Ivermectin on the microfilaria larva of *Simulium yahense*

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ABSTRACT

A comparative in vivo metabolic extracts of M.koenigii (seed), Citrus paradisi, (seed) and the leaves stem barks and roots of Alstonei boonei, Althemanthera repens, Ecliptra prostrata and Rothmannia longiflora and their combination were compared with Ivermectin against the third infectious microfilaria larva of Simulium yahense. The result showed remarkable larvacidal properties for ivermectin and the plant species under study as they could induce significant mortalities at low and varying concentrations in the larva of S. yahense. The LD_{50} and LD_{90} values estimated for M. koenigii, Citrus paradisi, Alstonei boonei, Althemanthera repens, Ecliptra prostrata, Rothmannia longiflora, their combination and Ivermectin are 7.24 and 15.49, 3.72 and 15.92, 2.92 and 7.32, 3.02 and 12.98, 3.02 and 14.87, 2.47 and 6.61 and 2.43 and 5.37 respectively. The study revealed that Ivermectin, Althemanthera repens and the combination of the plant extracts could induce 100% mortality of the larva at 6.0, 9.0, 12.0 and 15.0mg/Kg respectively.

Key words: Combination, Essential oil extracts, Ivermectin, onchocerciasis, Simulium yahense.

INTRODUCTION

Onchocerciasis is an infestation with filarial worms of the genus Onchocerca, common in tropical America and Africa, transmitted by black flies, and characterized by nodules under the skin, an itchy rash, eye lesions, and in severe cases, elephantiasis. The principal clinical manifestations are ocular lesions, resulting in visual, impairment and blindness, and onchocercal skin disease (OSD) (Murray and Lopez 1996). Szirmai, 2005 estimated that West Africa share about 30% of global cases of onchocerciasis with 96% of reported cases globally occurring in Africa. More so, this disease burden has contributed significantly to increased incidence of eye defects in tropical West Africa. For example, Dadzie 1989 reported an onchocerciasis prevalence ratio of 1:3 to other ocular problems in north central and north eastern part of Nigeria. Like most other diseases, majority of infestation cases are hardly reported nor documented. Over the years, control and management of the larvacidal activities of this epidemic has been achieved with diethyl carbazamine and most notably with Ivermectin. Ivermectin marketed under the brand name Stromectol is administered orally and available in 3-mg tablets containing microcrystalline cellulose, pregelatinized starch, magnesium separate, butylated hydroxyanisole, and citric acid powder (anhydrous). It is derived from the avermectins, a class of highly active broad-spectrum, anti-parasitic agents isolated from the fermentation products of *Streptomyces avermitilis*. It is a white to yellowish-white, nonhygroscopic, crystalline

powder with a melting point of about 155°C. It is insoluble in water but is freely soluble in methanol and soluble in 95% ethanol. The required dosages for patients between ages 15-25 , 26-44 , 46-64,65-84 and above 84 are one, two ,three, four and 150mcg/kg of 3mg tablets respectively. However, clinical studies have shown that STROMECTOL (ivermectin) has no activity against adult Onchocerca volvulus parasites since they reside in subcutaneous nodules which are infrequently palpable. Surgical excision of these nodules (nodulectomy) is often considered in the management of patients with onchocerciasis, since this procedure will eliminate the microfilaria-producing adult parasites (Ali *et al.*, 2003). More so, the use of this drug is fraught with vision changes, urinary or bowel problems, weakness, confusion, lack of coordination, eye redness, swelling, or pain; or seizure (convulsions) (De Sole, *et al*., 1989 and De Sole *et al*., 1991). It is in view of these shortcomings, that researches using cheap, readily available and negligible side effects from natural plant products are being championed by the Federal Ministry of Health in Nigeria. Evidences show that the number of people who rely on natural plant products even in United States is increasing (Mikaili et al., 2011**).** At the moment, priority is being paid to the application of these natural plant products through the use of lavicides and ovicides as ideal control measures.Natural products and secondary metabolites formed by living systems, notably from plant origin, have shown great potentials in treating human diseases such as cancer, coronary heart diseases, diabetes, malaria and infectious diseases. It is against this back drop that this study aims at evaluating the larvacidal properties of these six plant species, their combination and Ivermectin against the infectious larva stage of *Simulium yahense.* In the present investigation, specific action of each plant, their combination and Ivermectin were evaluated against the third infectious stage (microfilaria) of *Simulium yahense*.

MATERIALS AND METHODS

Sample Collection

Six 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.

Ivermectin was obtained as a classified drug with No. 8495 and supplied as NDC 0006-0032-20, with unit dose packages of 20, storage temperature below 30°C (86°F) and an expiring shelf life of 06-09-2012. STROMECTOL (ivermectin) 3 mg are white, round, flat, bevel-edged tablets coded MSD on one side and 32 on the other side, distributed by MERCK & CO., INC, Whitehouse Station, NJ 08889, USA and manufactured by MSD BV Waarderweg 39 2031 BN Haarlem Netherlands.

Methods

The seeds of *M.koenigii*, *Citrus paradisi* and the leaves stem bark and roots of *Alstonei boonei*, *Althemanthera repens, Ecliptra prostrati and Rothmannia longiflora* were sun-dried at ambient temperature (300c). After drying, 1kg each of the samples was pulverized to coarse powder using sterile mortar and pestle. Three hundred grams each of the pulverized herbs were measured electrically and transferred into a conical flask. Fifty grams each of the six herbs were weighed and mixed together to yield 300grams of the combined herbs and transferred into the seventh flask. The respective herbs and their combinations were extracted for 72hours using 1L of 98% ethanol. The herb-ethanol mixtures were shaken daily to ensure proper extraction. After 72hrs, the extracts were filtered, using clean white cotton. The filtrates were concentrated under vacuum in a rotary evaporator to yield 2.438, 2,745, 2.314, 4.311 3.845, 3.362 and 6.497g of sticky oily residues with moderate to low viscosity of *M. koenigii*, *C. paradisi*, *A boonei, A. repens, E. prostrata and R. longiflora* and their combination respectively. They were stored in screw capped vials at laboratory conditions until tested. Prior to use, 1g/ml of each extract was prepared in ethanol to ensure adequate dissolution and then 4ml of sterile water was added to give a stock of 1000mg in 5ml. Subsequently, 3.0, 6.0, 9.0, 12.0 and 15.0mg/kg doses were prepared using distilled water. The extraction was carried out n the department of Biochemistry, UNICAL.

Mouse strain

Healthy albino mice, four weeks old and weighing 15-22kg was obtained from the animal house of Genetics and Biotechnology, UNICAL. They were kept in cages and fed with livestock feeds and water for optimal health. Before the expt., the animals were bred for two weeks at botany research lab, UNICAL, where the research took place for proper acclimatization.

Innoculation and treatment

The microfilaria larvae were prepared and obtained from Nigeria Institute for pharmaceutical research and development, Idu, Abuja. Pure culture of the larva containing 25 microfilaria each were prepared and stored in glass vials at 27 ± 0.2^0C . The larvae were provided a mixture of Dog biscuit, yeast powder and algae in a ratio 3:1:1 ratio. On day 0, experimental as well as control groups of animals were inoculated 25 microfilaria larvae. The mice were then randomly divided into groups of three per cage and groups of experimental animals were given doses (3.0, 6.0, 9.0, 12.0and 15.0 mg/Kg) of test material after day 1. One other group received either one, two, three, four or five-3mg/kg ivermectin grounded tablet (positive control) and the other group received 0.2Ml of Phosphate Buffer Saline(PBS){ concentration of 137mM NaCl, 10Mm Phosphate, 2.7Mm KCl and Ph 7.4(negative control)}. Three replicates were run for each concentration. On day seven of the test, thin blood smears were prepared using well labeled and properly cleaned slides. Blood was collected from the eye vein of each animal. The dry blood films were fixed with methanol and subsequently stained with Geimsa for 30minutes. They were then washed in phosphate buffer, pH 7.2 and allowed to dry. To ensure optimal film quality, each slide was prepared in duplicate and mortally counts were made after 24hours exposure. Test showing more than 15% control mortality was discarded and repeated. However, when negative 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 LD50 and LD90, and the heterogeneity values, (Finney 1952).

RESULTS AND DISCUSSION

The result of the study indicated that in vivo methanol extracts of *M.koenigii, Citrus paradisi* and the leaves stem barks and roots of *Alstonei boonei, Althemanthera repens, Ecliptra prostrate, Rothmannia longiflora, their combination and ivermectin displayed a very good activity against microfilaria larva of Simulium yahense when given at doses of 3.0, 6.0, 9.0, 12.0 and 15.0mg/Kg respectively. This was evident in the LD50 and LD90 values shown in table 1.*

Table 1: Dosage response of the essential oil of six plant species, their combination and Ivermectin against the microfilaria larvae of *Simulium yahense*

Extract	LD50 values(ppm)	LD90 values(ppm)	Heterogeneity values-Chi-square test(df)*	Relative toxicity
Citrus paradisi	7.24	15.49	23.58 (3)	3:1
Murraya koenigii	8.71	16.42	53.37 (3)	3:1
Alstonei boonei	3.72	15.92	21.81 (3)	2:1
Althemanthera repens	2.92	7.32	5.86 (3)	1:1
Ecliptra prostrata	3.02	12.98	29.58 (3)	1.5:1
Rothmannia longiflora	3.02	14.87	9.98 (3)	1.5:1
Combination	2.47	6.61	7.26 (3)	1:1
Ivermectin	2.43	5.37	7.04 (3)	

Ppm: parts per million; LD50: lethal concentration needed to kill 50% of larva exposed; LD90: lethal concentration needed to kill 90% of larva exposed; *df: degree of freedom; $x^{2:}$ chi-square for heterogeneity

The comparison analysis indicated that Combination of the plant extracts, *Althemanthera repens, Ecliptra prostrata and Rothmannia longiflora displayed remarkable lavacidal activity at very low dosage similar to that shown by ivermectin. Also the calculated chi-test values of these extracts revealed that the differences were insignificant and due to chance alone. Similarly, 100% mortality was observed at all doses, save 3.0mg/Kg among Ivermectin,* Combination *and Althemanthera repens respectively. This similarity in lavacidal action was evident in the relative toxicity values of 1:1 to ivermectin. Other extracts were shown to be either twice or thrice as effective as ivermectin or* Combination *or Althemanthera repens with the exception of Ecliptra prostrata and Rothmannia longiflora which showed less than twice as effective as Ivermectin or Combination extract or Althemanthera repens.*

Previous literatures on lavacidal control of microfilaria have been carried out through spraying of endemic breeding sites over a long-distant range (Remme, 2004). Other control measures include, epidemiological modeling (Remme, *et al*., 1995), research on disease patterns and community trials of Ivermectin, (WHO, 1995).Such control measures are either subjective, environmentally hostile or fail to achieve target objective. There have been dearths of published works on the use of natural products of plant origin in the larvacidal control of onchocerciasis.

CONCLUSION

This study indicates that the essential oil extract of *M.koenigii*, *Citrus paradisi*, and the the leaves, stem barks and roots of *Alstonei boonei*, *Althemanthera repens*, *Ecliptra prostrata and Rothmannia longiflora* and their combination and Ivermectin has remarkable lavacidal activity against *Simulium yahense.* It is noteworthy to study extensively the lavacidal properties of the essential oils contained in the plants by isolating and identifying the active ingredients that cause larval mortality and then use them in field trials in order to assess their potentials as alternative to ivermectin and other chemical larvacides whose activities are being reviewed.

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