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Effects of *Moringa oleifera*, A Plant Extract Coded OBAYOKOU on Ulcers Caused by *Mycobacterium ulcerans* In Children under 15 Years in Côte d'Ivoire

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ABSTRACT
The aqueous extract of a plant coded OBAYOKOU (*Moringa oleifera*) has been tested on the healing of ulcers in children under 15 years, two lots of 15 children each were made all presenting clinical forms of Buruli ulcer. The aqueous plant extract of *Moringa oleifera* was added to the food supply given to Lot B (lot of experimental subjects) at a rate of 330 ml per meal per child for six weeks. Children of Lot A (control Lot) received normal diet without *Moringa oleifera*. The results of this study have shown that children in (lot B) in addition to the normal diet received 330 ml of *Moringa oleifera* per meal, had a higher rate of healing than Lot A (Witness Lot) who received only normal diet. Thus, the rate of healing of ulcers with *M. oleifera* (24 mm) is superior to healing without *M. oleifera* (10 mm) after six weeks. This study shows that we can overcome Buruli ulcer with medicinal plants that is abundantly available in Africa. It also shows that it is necessary to conduct research on a wider range of fractions of *Moringa oleifera* to improve the activity of antimycobacteriennes molecules in the aqueous extract of *Moringa oleifera*.

Keywords: Buruli ulcer, *Mycobacterium ulcerans*, *Moringa oleifera*, Côte d'Ivoire.

INTRODUCTION

Buruli ulcer (Bu) or *Mycobacterium ulcerans* is a human skin infection caused by mycobacteria present in the environment. This is the most encountered mycobacterial disease currently after tuberculosis. [1] *M. ulcerans* infection, leads to extensive damage of skin and soft tissues with the formation of large ulcers usually on the limbs causing deaths in few cases, this disease is causing significant functional disability. **[2]** This disease affect mostly children from 2 years in more than 50% of cases, it affects children under the age of 15 years regardless of sex. In adult patients Buruli ulcer affects women more often than men. [1, 3-4] The mode of transmission of *M. ulcerans* is still subject of controversy. Although there is no direct transmission from man to man, a link is generally recognized with contact with stagnant water, so that the existence of a hydrotelluric reservoir is strongly suspected. [5-6] Since 1980, there has been a dramatic increase in cases of Bu in the world. [7-8] In sub-Saharan Africa children and women are more often affected than men. In these areas, the prevalence is much higher especially along the Gulf of Guinea where the disease is rapidly expanding. [9] The three major endemic countries are Ghana, Benin and Côte d'Ivoire. Every year, health care centers and Bu disease management centers record a number of increasingly new cases in Côte d'Ivoire. [8] Disease management duration is very long and expensive therefore inaccessible to most of the patients.

This worrying situation is a real and serious public health problem in Côte d'Ivoire. [9-10] WHO recommends association of antibiotics as first-line treatment and surgery as a second-line treatment for Buruli ulcer. But we noted that in both cases, there are cases of relapses. [1] Traditional medicine is readily used by the population both as curative and preventive purposes. [11-12] Medicinal plants are used in the treatment of many diseases (digestive diseases, oral, lung,

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liver and skin etc ...). [11-13] In order to contribute to the search for a cure against Buruli ulcer, the aqueous plant extract of a plant coded OBAYOKOU (*Moringa Oleifera*) has been tested on the healing of ulcers in children under 15 years by adding it to their diet for six weeks.

MATERIALS AND METHODS

Plant material

The plant material consisted of the solution from fresh plant leaves harvested from flowering *Moringa oleifera*. This plant has been identified by a specialist from the National Floristic Center Côte d'Ivoire, where sample are kept.

Place of study

The study was conducted in one of the Buruli ulcer patients management and caring (Centre St Pio Padio) centers. This center is located in Abidjan, Côte d'Ivoire.

Target population

They are children who presented skin lesions clinically suggestive of Buruli ulcer. Age range of 2-15 years and this study was conducted on a population of 30 children divided into two groups of 15 children per lot.

Preparation of the plant extracts

The fresh leaves are harvested from flowering *Moringa* plant and disinfected in 10 g of potassium permanganate for 20 min. These leaves are then rinsed and soaked in 500 ml of tap water. They were then crushed; we obtained a greenish solution it was allowed to settle, away from the light. The extract was filtered in BÜCHNER with Whatman 3mm paper. In the filtrate obtained were added 10 cubes of sugar and the volume of this filtration is brought to 1000 ml by adding tap water.

Experimental approach

This experimental study was conducted on sample of 83 children admitted to the center all having clinical forms of the disease. The informed consent of parents or legal guardians should be obtained prior to inclusion in the study. The selection has allowed us to select thirty (30) children divided into two groups A and B of 15 children each. Group A (Witness group) involved children who received normal diet while group B (subjects) children received a normal diet to which was added 330 ml of *Moringa oleifera* at each meal per child. This experiment was conducted for six weeks. At the end of each week; children ulceration were measured in both lots with a ruler.

Statistical Analysis

To process data, we used the statistical law of Fisher-Snedecor based on the comparison of variances of the two groups. Working at the simplest level, we have computerized and analyzed survey data to produce cross, medium and graphics tabs.

RESULTS

Ulceration measure in children of Lot A, group receiving diet without *Moringa oleifera*

Lot A (Witness) consists of children who received a normal diet without *Moringa oleifera*. Healing of ulcers during the six weeks increased from Do (66mm) to D_{56} (56mm) (Table I and Figure 1).

Ulceration measure in children of Lot B, normal diet with *Moringa oleifera*

Children of Lot B (experimental subjects) received in addition to a normal diet, an amount of 330 ml of *Moringa oleifera* at every meal. Ulcers measure in children of this lot reduced from Do (72mm) to D_{56} (48mm) (Table II and Figure 1).

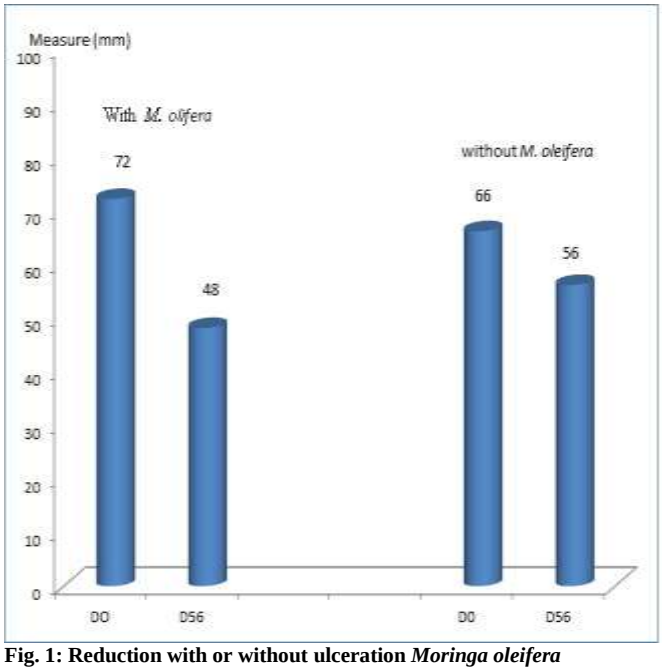

Fig. 1: Reduction with or without ulceration *Moringa oleifera*

Table I: Ulceration measures in children of lot A for six weeks

N° of patient	Ulceration at D_0 in (mm)	Ulceration at D_{56} in (mm)	Reduction in ulceration (mm)
1A	55	47	08
2A	115	106	09
3A	195	187	08
4A	121	109	12
5A	175	NF	NF
6A	23	16	07
7A	25	15	10
8A	14	08	07
9A	135	125	10
10A	20	08	12
11A	30	17	13
12A	66	55	11
13A	16	07	09
14A	65	52	13
15A	35	25	10
Moyenne	**66**	**56**	**10**

NF: not measured D_0 : First day D_{56} : 56th day

Table II: Ulcers measure in children of Lot B for six weeks

N° of patient	Ulceration at D_0 in (mm)	Ulceration at D_{56} in (mm)	Reduction in ulceration (mm)
1B	78	53	25
2B	155	NF	NF
3B	170	150	20
4B	45	21	24
5B	67	35	32
6B	55	34	21
7B	162	146	16
8B	39	15	24
9B	45	26	19
10B	30	12	18
11B	46	18	28
12B	34	13	21
13B	50	26	24
14B	150	NF	NF
15B	105	75	30
Moyenne	**72**	**48**	**24**

NF : not measured D_0 : First day D_{56} : 56th day

DISCUSSION

The aim of our study was to evaluate the effects of active principles contained in the aqueous extract of *Moringa oleifera* on ulcerations caused by *Mycobacterium ulcerans* in children under 15 years in Côte d'Ivoire for six weeks. This plant is used in traditional medicine to treat incurable

wounds. The results of this study showed that children who were given at each meal 330 ml of *Moringa oleifera* in addition to the normal diet, had a higher rate of healing than children in Lot A who received only normal diet without *Moringa oleifera*. Healing ulcers with *M. oleifera* (24 mm) > Healing ulcers without *M. oleifera* (10 mm). These results showed that *M. oleifera* has antimycobacteriennes molecules. Our results were confirmed by several research teams who worked on the active principle contained in medicinal plants**.** [1]

In 2013, the effect of aqueous extract of PHYLAM (*Phyllanthus amarus*), a plant used in traditional medicine circle to treat incurable wounds was evaluated on the *in vitro* growth of two strains of *M. tuberculosis* (H37Rv and RF94) and one strain of *M. ulcerans* (02003). The results showed that the three strains of mycobacteria were sensitive to the aqueous extract of *P. amarus*, in dose-response relationship. [**8**] These results had been reported in Côte d'Ivoire in 2006 by study on the evaluation of the activity of aqueous and ethanolic extracts of *P. amarus* on the *in-vitro* growth of seven strains of *Mycobacterium ulcerans* isolated from different regions of the country. These aqueous and ethanolic extracts of *P. amarus* exerted effective inhibitory activities on strains tested at 32 mg / ml. [14] The bactericidal action of medicinal plants was reported in 2014 by the work carried out on the *in-vitro* evaluation of total flavonoids of *Thonningia sanguinea* against three strains of *Staphylococcus genus*, namely a reference strain (*Staphylococcus aureus* ATCC 25923) and two clinical strains (*Staphylococcus aureus methicillin* –S and *Staphylococcus aureus methicillin -R*). The results revealed that the total flavonoids of *Thonningia sanguinea* have bactericidal activity on three strains of the genus Staphylococcus tested. [15] These same results were observed in 2007 and in 2011 by the study conducted on the *in-vitro* evaluation of ethanolic extracts of *Thonningia sanguinea* and extract of *Terminalia glaucescens* on *Cryptococcus neoformans* and *Salmonella typhi* respectively. [16-17] These observations showed that our continent is well endowed with varieties of plants that contain substances that inhibit mycobacteria. This justifies the use of plants in traditional medicine in the treatment of several pathologies. [12]

In this study, we evaluated the effects of the active principles contained in the aqueous extract of *Moringa oleifera* on ulcerations caused by *Mycobacterium ulcerans* in children under 15 for six weeks. The results of this study showed that *Moringa oleifera* contain antimycobacteriennes molecules. This could help to find a cure against this disease that is a real public health problem in Côte d'Ivoire.

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