FINAL REPORT REPORT

July 2007 - May 2008

A comparative investigation of the anti fungal properties of indigenous wild thyme *spp*. with locally distilled essential oils

1. Introduction

The following final report is made up of three parts. *Section I* presents a briefing of all the activities accomplished with relevant results in line with project proposal. *Section II* deals with the official presentation of the clinical results of the trial accomplished at school level in order to evaluate the efficacy of the cream based on wild collected thyme from which essential oil was locally distilled. Finally *Section III* offers some remarks and conclusion about the project accomplishment.

Thyme based cream tested by IPO on Tinea (mycotic infections of skin) effectively better skin condition on **89,8%** of the cases. Out of this, **66,4% was completely cured** while 23,5% had a good improvement. Together with the cream, an ointment against ringworm (Tinea *capitis*) was tested out. Ointment improved **77,8%** of the cases. **43,6% of children was successfully cured** while a partial improvement was observed in 28,9% and a very good improvement in 5,26%.

2. Section I

2.1 Summary of achievements prior to last quarter

- ✓ Potential areas identified and further contacts taken with local administrations and communities in order to verify the interest in the concerned activities.
- ✓ Sites visited and fresh sample of Thymus *schimperi* collected and afterwards distilled. A total of 9 samples distilled after being dried under different processes and conditions.
- ✓ Samples delivered to Urbino University for Gc/Ms analysis to determine the essential oil contents and main constituents which characterize the specific 'chemiotype'.
- ✓ Identification of thymol/carvacrol chemotype.
- ✓ Confirmation on drying best practices (1 month not in direct sunlight).
- ✓ Special training on thyme distillation techniques to a local cooperative involved into distillation Tirign Aromatic Oil Cooperative, Addis Abeba.
- ✓ Air drying apparatus delivered to Tirign Aromatic Oil Coop.
- ✓ Choice of essential oil (not less than 40% of phenolic compounds accordingly with F.U.I).

- ✓ Cream formula elaboration.
- ✓ Pavia University accomplished patch tests with non irritating final evaluation (five different patch tests were undertaken each one to 25 healthy and well-informed on the test purpose volunteers).
- ✓ 60 kg of dried leaves distilled producing a total amount of about 1 litre of essential oil.
- ✓ Cream preparation training for Tirign Aromatic Oil Cooperative.
- ✓ Ointment and cream prepared.
- ✓ Dr. Vitalia Murgia (IPO scientific committee), 'Therapeutic potentialities of some Ethiopian essential oils on mycotic skin diseases' presented at the Second International Congress '*Dermatological care for all: a basic human right*', Addis Abeba-Mekele, Ethiopia, Novembre, 2007.
- ✓ Link with local dermatologist Dr. Kassahun Bilcha for clinical trial
- ✓ Area and school selection in Debre Markos
- ✓ Zonal administration permission and ethical clearance

3. Section II

Prior to the clinical trial, an epidemiological study was carried out to rationally include in the study children with targeted pathologies, following an inclusion/exclusion criterion. After that, selected children were re-examined and medicated with 3 types of drugs. Children with Tinea *capitis* infection (ringworm) were randomly treated with IPO ointment or weekly Fluconazole according to their weight. Children with Tinea *corporis* were treated using IPO cream. Both treatments based on essential oil were apply to children to whom a lesson on how and where to apply was given together with focus on importance of compliance with treatment and care for hygiene. Weekly Fluconazole was administered by a nurse as a systemic drug (tablet) after indication were found in literature of effectiveness of the drug once a week for a month course. The nurse was checking the students at school daily and record the compliance of each patient.
Herein Chapter 3.1 presents general epidemiological data which were used to select patients. Chapters from 3.1 to 3.4 present findings of the project and highlight results. Specifically, Chapter 3.2 discusses about IPO cream findings, while subsequent 3.3 deals with IPO Ointment and 3.4 with weekly Fluconazole trial.

3.1 Debre Markos study general data

During the study **1102** children were screened in two different Debre Markos schools namely Wonka and Geterfana in order to assess their general skin health condition. The overall frequency of children with skin disease or skin condition was as high as **74%** (see table 6). We focused our attention on those skin diseases that are know to be more common in children. We registered a very poor hygiene condition in more than 98% of the children. More of them weren't properly dressed and protected from cold or for any aggressive agent. They were generally barefoot and they had, all over the body, a lot of scars. Some of these scares are burning result and attest that the children could be very frequently exposed at very hazardous situation without parents' control.

Table 1 shows the number of children screened per each school.

Table 1

School	Frequency	Per cent	
Geterfana	636	57,6%	
Wonka	468	42,4%	
Total	1104	100,0%	

Table2 shows the frequency of children per gender

Table 2

Gender	Frequency	Per cent	
Female	555	50,3%	
Male	549	49,7%	
Total	1104	100,0%	

The mean age of the children was ten year, with a maximum age of 19 and a minimum of seven year. Table 3 shows the frequency of the different ages.

Table 3

Age	Frequency	Per cent	
7	134	12,2%	
8	248	22,5%	
9	119	10,8%	
10	259	23,5%	
11	132	12,0%	

12	101	9,2%
13	52	4,7%
14	34	3,1%
15	20	1,8%
16	2	0,2%
19	1	0,1%
Total	1102	100,0%

Table 4 shows the classroom frequency distribution of the screened children in Wonka school

Table 4

Classroom	Frequency	Percent
1A	36	7,7%
1B	34	7,3%
1C	34	7,3%
1D	35	7,5%
2A	33	7,1%
2B	36	7,7%
2C	19	4,1%
3A	47	10,0%
3B	51	10,9%
4A	46	9,8%
4B	48	10,3%
4C	48	10,3%
4D	1	0,2%
Total	468	100,0%

Table 5 shows the classroom frequency distribution of the screened children in Geterfana school

Table 5

Classroom	Frequency	Percent
1A	55	8,6%
1B	45	7,1%
1C	55	8,6%
1D	39	6,1%
2B	57	9,0%
2C	46	7,2%
3A	51	8,0%
3B	45	7,1%

3C	46	7,2%	
4A	52	8,2%	
4B	54	8,5%	
4C	45	7,1%	
4D	46	7,2%	
Total	636	100,0%	

Table 6 shows the overall incidence of skin diseases

Table 6

Debre Markos Study (1102 children)							
Disease	**Total**	**Wonka**	**Geterfana**	**Female**	**Male**	**Age<8**	**Age>8 and <12**
	n=1102	n=468	n=736	n=555	n=449	n=382	n=611
Dermathological problems	816 (74,0%)	320 (68,7%)	496 (78%)	417 (75,3%)	399 (72,8%)	294 (77%)	446 (73,2%)
Tinea infections	399 (36,1%)	174 (37,2%)	225 (35,4%)	150 (27,1%)	249 (45,4%)	161 (42,1%)	214 (35%)
Tinea capitis	305 (27,6%)	112 (23,9%)	193 (30,3%)	111 (20%)	194 (35,3%)	130 (34%)	163 (26,7%)
Tinea corporis	107 (9,7)	58 (12,4%)	49 (7,7%)	31 (5,6%)	76 (13,8%)	45 (11,8%)	57 (9,3%)
Tinea unguium	35 (3,2%)	17 (3,6%)	18 (2,8%)	23 (4,1%)	12 (2,2%)	10 (2,6%)	19 (3,1)
Head lice	345 (31,2%)	122 (26,1%)	223 (35,1%)	250 (45%)	95 (17,3%)	119 (31,2%)	201 (32,9%)
Wart	95 (8,6%)	33 (7,1%)	62 (9,7%)	41 (7,4%)	54 (9,8%)	30 (30,9%)	56 (9,2%)
Pityriasis alba	49 (4,4%)	11 (2,4%)	38 (6,0%)	21 (3,8%)	28 (5,1%)	18 (4,7%)	26 (4,3%)
Others	39 (3,5%)	11 (2,4%)	28 (4,4%	22 (4%)	17 (3,1%)	6 (1,6%)	11 (1,8%)
Pyoderma	29 (2,6)	13 (2,8%)	16 (2,5%)	15 (2,7%)	14 (2,6%)	10 (2,6%)	16 (2,6%)
Eczema	23 (2,1%)	9 (1,9%)	14 (2,2%)	12 (2,2%)	11 (2,0%)	12 (3,1%)	7 (1,1%)
Nevus	43 (3,9%)	16 (3,4%)	27 (4,2%)	23 (4,1%)	20 (3,6%)	10 (2,6%)	28 (4,6%)
Tubercolosis	3 (0,3%)	1 (0,2%)	2 (0,3%)	0	3 (0,3%)	0	2 (0,3%)
Herpes Simplex	10 (0,9%)	6 (1,3%)	4 (0,6%)	1 (0,2%)	9 (1,6%)	2 (0,5%)	6 (1%)
Molluscum contagiosum	3 (0,3%)	1 (0,2%)	2 (0,4%)	2 (0,4%)	1 (0,2%)	2 (0,5%)	1 (0,2%)

Table 6 (continues)

Disease	Total	Wonka	Geterfana	Female	Male	Age<8	Age>8 and <12
Scabies	2 (0,2%)	2 (0,4%)	0	1 (0,2%)	1 (0,2%)	0	2 (0,3%)
Psoriasis	1 (0,1%)	1 (0,2%)	0	1 (0,2%)	0	1 (0,3%)	0
Urticaria	4 (0,4%)	1 (0,2%)	3 (0,5%)	2 (0,4%)	2 (0,4%)		0
Photodermatosis	0	0	0	0	0	0	0
Vitiligo	0	0	0	0	0	0	0

3.2 IPO Cream Trial

Table 7 shows the total number of patients included into the assessment for Tinea *corporis* (9,7% of the total).

Table 7

School	Frequency	Percent	
GETERFANA	46	45,5%	
WONKA	55	54,5%	
Total	101	100,0%	

Table 8 shows the frequency of children per gender

Table 8

Gender	Frequency	Percent	
Female	31	30,7%	
Male	70	69,3%	
Total	101	100,0%	

The mean age of the children was eleven year, with a maximum age of 16 and a minimum of seven year. Table 9 shows the frequency of the different ages.

Table 9

Age	Frequency	Percent	
7	11	10,9%	
8	32	31,7%	
9	9	8,9%	
10	24	23,8%	
11	14	13,9%	
12	6	5,9%	
13	3	3,0%	
14	1	1,0%	
16	1	1,0%	
Total	101	100,0%	

Table 10 shows the number of drop out (7 children were absent the day of the final assessment and 5 didn't apply the cream with the planned schedule)

DROP OUT	Frequency	Percent	
Yes	5	5,3%	
No	89	94,7%	
Total	94	100,0%	

Table 11 shows the initial situation of skin lesions with majority classified as moderate

Table 11

Baseline global Assessment of the lesions (extension and entity)	Frequency	Percent	
mild	26	25,7%	
moderate	62	61,4%	
severe	13	12,9%	
Total	101	100,0%	

Table 12 shows the final situation after 24 days of cream application (considering 5 drop out and 7 absentees) . 66,3% are classified as 'nothing' which stands for completely cured

Table 12

Final global assessment of the lesions (extension and entity)	Frequency	Percent	
Mild	26	29,2%	
Moderate	2	2,2%	
nothing	59	66,3%	
Severe	2	2,2%	
Total	89	100,0%	

Fig 2 shows the child before treatment with typical T. *corporis* lesions in the face (right eye brow, nose, right cheek)

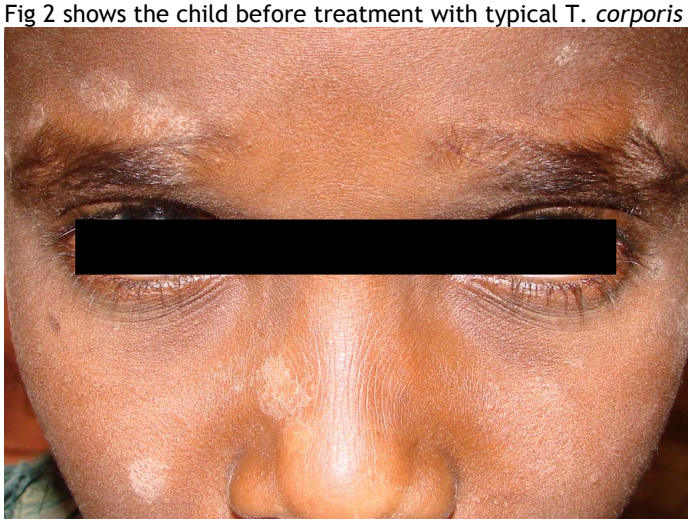

Fig 3 shows the same child after 24 days of treatment

Fig 4 shows a child before and after the treatment (lesions are on the forehead)

Table 13 shows doctor's judgement on efficacy with 91% of good and above

Table 13

Efficacy	Frequency	Percent	
very low	2	2,2%	
low	1	1,1%	
sufficient	5	5,6%	
good	22	24,7%	
very good	59	66,3%	
Total	89	100,0%	

Table 14 shows patients' judgement on efficacy with similar results than doctor's one

Table 14

Child's Global assessment of treatment	Frequency	Percent	
Low	1	1,1%	
Sufficient	5	5,6%	
Good	21	23,6%	
Very good	62	69,7%	
Total	89	100,0%	

Table 15 and 16 present respectively doctor and children assessment of tollerability which confirm the patch test result (non irritating)

Table 15

Doctor's assessment of tollerability	Frequency	Percent
Good	1	1,1%
Very good	88	98,9%
Total	89	100,0%

Table 16

Child's assessment of tollerability	Frequency	Percent
Very low	2	2,2%
Low	1	1,1%
Good	9	10,1%
Very good	77	86,5%
Total	89	100,0%

Table 17 shows no adverse reactions were detected in patients

Table 17

Product sensitization	Frequency	Percent
No	89	100,0%
Total	89	100,0%

Fig 1 shows the patch tests results

Fig 1

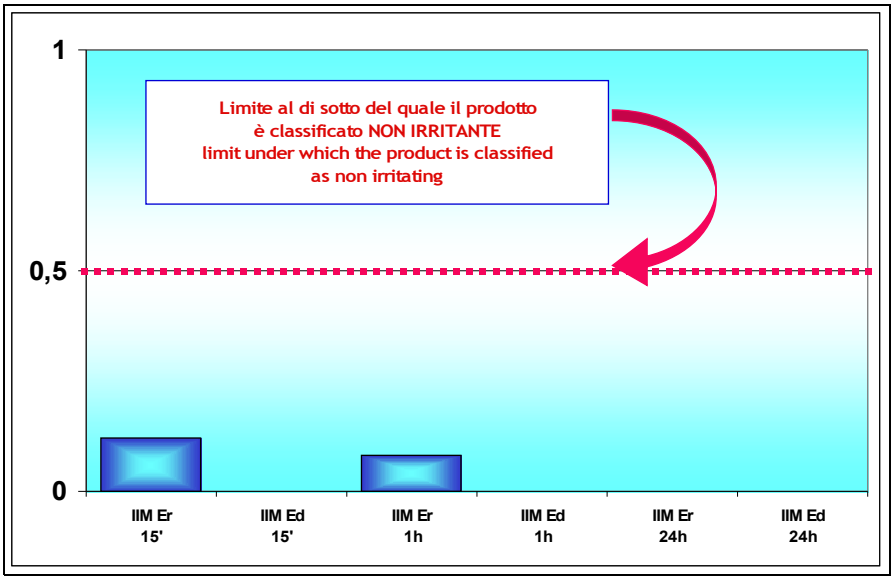

Table 18 shows the frequency of lesions classified by their seriousness out of the 66,3% cured children. The fact that most of the lesions were moderate demonstrates a good degree of efficacy of the cream. It is thinkable that an almost 100% level of cured patients could be accomplished if lesions were treated in time giving an high potentiality to the cream to be a medical praesidium and an useful tool for teaching prevention and to avoid worsening of skin disease.

Table 18

CURED CHILDREN Baseline global assessment of the lesions (extension and entity)	Frequency	Percent	
mild	18	30,5%	
moderate	38	64,4%	
severe	3	5,1%	
Total	59	100,0%	

Table 19,20,21 show the percentage of response out of the children with mild, moderate and severe lesions. It is clear that 23,5 % have had an improvement out of which 15,7% had a medium improvement but 7,8% an optimum one. This consideration lead us to think that under better compliance conditions or simply prolonging the treatment for the needed cases the percentage of complete healing could be 90%.

Table 19 Percentage of response out of the mild children (24/89)

Final global assessment of the lesions (extension and entity)	Frequency	Percent	
nothing	18	75,0%	
mild	6	25,0%	
Total	24	100,0%	

Table 20 Percentage of response out of the moderate children (55/89)

Final global assessment of the lesions (extension and entity)	Frequency	Percent	
nothing	38	69,1%	
mild	15	27,3%	
moderate	1	1,8%	
severe	1	1,8%	
Total	55	100,0%	

Table 21 Percentage of response out of the moderate children (10/89)

Final global assessment of the lesions (extension and entity)	Frequency	Percent	
nothing	3	30,0%	
mild	5	50,0%	
moderate	1	10,0%	
severe	1	10,0%	
Total	10	100,0%	

3.3 IPO Ointment trial

Table 22 shows the total number of patients included into the assessment for Tinea *capitis* (27,6% of the total). The children were randomly selected (one out of 5 in the computer generated list) in two different schools: Geterfana and Wonka.

Table 22

School	Frequency	Per cent	
GETERFANA	28	66,7%	
WONKA	14	33,3%	
Total	42	100,0%	

Table 23 shows the frequency of children per gender

Table 23

Gender	Frequency	Per cent	
Female	14	33,3%	
Male	28	66,7%	
Total	42	100,0%	

Table 24 shows the range of age of the children

Table 24

Age	Frequency	Per cent	
7	5	11,9%	
8	11	26,2%	
9	4	9,5%	
10	12	28,6%	
11	5	11,9%	
12	3	7,1%	
14	1	2,4%	
15	1	2,4%	
Total	42	100,0%	

Table 25 and 26 show the number of drop out (2 children were absent the day of the final assessment) and number of who didn't apply the cream with the planned schedule

Table 25

DROP OUT	Frequency	Per cent	
Yes	1	2,6%	
No	38	97,4%	
Total	39	100,0%	

Table 26

No compliance	Frequency	Per cent	
Yes	2	4,8%	
No	40	95,2%	
Total	42	100,0%	

Table 27 shows what on the first day was assessed as general extension and entity of the lesions with a three steps score: mild, moderate, severe. Again 54,8% of the children had moderate score. Severe cases account for 19%. Social stigma is clearly visible from the picture.

Fig 4 How clearly recognizable it is a child with ringworm

Table 27

Baseline global assessment of the lesions (extension and entity)	Frequency	Per cent	
mild	11	26,2%	
moderate	23	54,8%	
severe	8	19,0%	
Total	42	100,0%	

Table 28 shows the frequency of cured 43,6% as well as, if compared with the above one, percentages of improvement

Table 28

Final global assessment of the lesions (extension and entity)	Frequency	Per cent
nothing	17	43,6%
mild	14	35,9%
moderate	6	15,4%
severe	2	5,1%
Total	39	100,0%

Table 29 shows the subjective doctor's assessment of the efficacy with 73,6% of positive result.

Table 29

Efficacy	Frequency	Per cent
very low	2	5,3%
low	2	5,3%
sufficient	5	13,2%
good	9	23,7%
very good	20	52,6%
Total	38	100,0%

Table 30 shows children perception on efficacy of the treatment. It is remarkable that children judgment is higher than doctor (97,3%) and it can implies that the care to which children where exposed increased their own assessment of efficacy.

Table 30

Child's Global assessment of treatment	Frequency	Per cent
Very low	1	2,6%
Good	14	36,8%
Very good	23	60,5%
Total	38	100,0%

Table 31,32,33 show very good pleasantness and tolerability with no adverse reaction to ointment

Table 31

Pleasentness	Frequency	Percent
Very Good	38	100,0%
Total	38	100,0%

Table 32

Tollerability	Frequency	Per cent	
Good	1	2,6%	
Very good	37	97,4%	
Total	38	100,0%	

Table 33

Product sensitization	Frequency	Percent	
No	42	100,0%	
Total	42	100,0%	

Table 34 shows the frequency of lesions classified by their seriousness out of the 43,6% cured children. No severe cases of Tinea *capitis* were cured with the ointment. This is because it is difficult for the ointment to penetrate into the follicles were fungal infection resides. It is indeed promising that applied in time the ointment could treat most of the mild to moderate cases avoiding a worsening and therefore being used as an hygienic tool.

Table 34

Global Assessment of the lesions (extension and entity)	Frequency	Percent	
mild	7	41,2%	
moderate	10	58,8%	
Total	17	100,0%	

Table 35,36,37 show the percentage of response out of the children with mild, moderate and severe lesions. It is clear that 34,2 % had an improvement out of which 28,9% had a medium improvement but 5,26% an optimum one. Under controlled condition and doctor advice this improvement could eventually turning into healing.

Table 35 Percentage of response out of the mild children (9/38)

Final global assessment of the lesions (extension and entity)	Frequency	Per cent	
nothing	7	77,8%	
mild	2	22,2%	
Total	9	100,0%	

Table 36 Percentage of response out of the moderate children (23/38)

Final global assessment of the lesions (extension and entity)	Frequency	Per cent	
nothing	10	43,5%	
mild	9	39,1%	
moderate	4	17,4%	
Total	23	100,0%	

Table 37 Percentage of response out of the severe children (6/38)

Final global assessment of the lesions (extension and entity)	Frequency	Percent	
mild	2	33,3%	
moderate	2	33,3%	
severe	2	33,3%	
Total	6	100,0%	

3.4 Fluconazole trial

Together with the experimental herbal drugs, generic drugs were bought and prescribed and given free of charge for children, in order not to leave untreated children with other pathologies encountered during the study. Apart from topical drugs, a systemic drug, a well known antifungal already used since several years was tried with different posology. The idea was that even being generic (produced in India) the cost of the standard treatment per child would be around 300 ETB (20 EURO) which is unbearable for most of the families. A weekly treatment was therefore tried following successful histories drawn from literature (GUPTA, DLOVA, TABORDA, MORAR, TABORDA, LYNDE, KONNIKOV, BORGES, RABOOBEE, SUMMERBELL, ADAM, HOFSTADER, ABOOBAKER, *Once weekly fluconazole is effective in children in the treatment of tinea capitis: a prospective, multicentre study*, British Journal of Dermatology 2000; 142: 965±968) and from same authors *Intermittent short duration therapy with Fluconazole is effective for tinea capitis* published in the same journal one year ahead (British Journal of Dermatology 1999; 141: 304±306).

Fig 5 How it looks a severe case of Tinea *capitis*

Table 38 shows the total number of patients included into the weekly Fluconazole trial.

Table 38

School	Frequency	Per cent	
GETERFANA	38	65,5%	
WONKA	20	34,5%	
Total	58	100,0%	

Table 39 shows the frequency of children per gender

Table 39

Gender	Frequency	Per cent	
Female	21	36,2%	
Male	37	63,8%	
Total	58	100,0%	

Table 40 shows the range of age of the children

Table 40

Age	Frequency	Per cent	
7	6	10,3%	
8	18	31,0%	
9	7	12,1%	
10	14	24,1%	
11	5	8,6%	
12	7	12,1%	
14	1	1,7%	
Total	58	100,0%	

Table 41 shows that no drop out cases were recorded

Table 41

DROP OUT	Frequency	Per cent	
No	55	100,0%	
Total	55	100,0%	

Table 42 shows the compliance for weekly Fluconazole tablets which can indicate that only 69% of the children could be taken as reliable because they accomplished the treatment.

Table 42

Fluconazol compliance	Frequency	Per cent	
25%	2	3,4%	
50%	1	1,7%	
75%	15	25,9%	
100%	40	69,0%	
Total	58	100,0%	

Table 43 shows what on the first day was assessed as general extension and entity of the lesions with a three steps score: mild, moderate, severe. Again 58,6% of the children had moderate score with relevant percentage of serious cases (25,9%). Left unattended Tinea *capitis* could turn into severe disease with a lot of discomfort into the daily life of the child associated with strong stigma.

Table 43

Baseline global assessment of the lesions (extension and entity)	Frequency	Per cent	
mild	9	15,5%	
moderate	34	58,6%	
severe	15	25,9%	
Total	58	100,0%	

Table 44 shows that only 27,3% had a complete recovery which surprisingly could be compared to IPO ointment (43,6%).

Table 44

Final global assessment of the lesions (extension and entity)	Frequency	Percent	
nothing	15	27,3%	
mild	21	38,2%	
moderate	17	30,9%	
Severe	2	3,6%	
Total	55	100,0%	

Table 45 shows doctor subjective judgement of efficacy

Table 45

Doctor's Efficacy	Frequency	Percent	
very low	4	7,3%	
low	9	16,4%	
sufficient	7	12,7%	
good	17	30,9%	
very good	18	32,7%	
Total	55	100,0%	

Table 46 shows patient's perception of efficacy

Table 46

Child's Global assessment of treatment	Frequency	Percent	
Very low	4	7,3%	
Low	5	9,1%	
Sufficient	5	9,1%	
Good	19	34,5%	
Very good	22	40,0%	
Total	55	100,0%	

Table 47, 48,49 show the percentage of response out of the children with mild, moderate and severe lesions. 45,5% of the children had an improvement whit optimum improvement for 7,3%. These figures account totally for 52,8% of children who showed improvements. If we sum up cured and improved patients we reach 80% which is really close to the ointment percentage (77,8%).

47 Percentage of response out of the mild children (9/55)

Final global assessment of the lesions (extension and entity)	Frequency	Per cent	
nothing	5	55,6%	
mild	3	33,3%	
moderate	1	11,1%	
Total	9	100,0%	

Table 48 Percentage of response out of the moderate children (32/55)

Final global assessment of the lesions (extension and entity)	Frequency	Per cent	
nothing	8	25,0%	
mild	14	43,8%	
moderate	10	31,3%	
Total	32	100,0%	

Table 49 Percentage of response out of the severe children (14/55)

Final global assessment of the lesions (extension and entity)	Frequency	Per cent	
nothing	2	14,3%	
mild	4	28,6%	
moderate	6	42,9%	
severe	2	14,3%	
Total	14	100,0%	

4. Section III

4.1 Conclusion

The project aimed at investigating several wild species of thyme to verify whether or not their essential oil combined in a state of art formula to create an applicable product (cream and ointment) would be effective once tested on children.

Fig 6 shows a close up of IPO tested cream and ointment

Clinical data and evidences were collected and presented above. IPO believes this study is only the tip of the iceberg, revealing that, if a scientific approach is taken into proper consideration, plant extracts could represent a viable alternative to high cost, often imported, synthetic drugs. This study confirm the vision of IPO following which phytomedicine could effectively means self-sufficiency and protection of native rights to benefit from their own natural resources.