



## In Vivo Trials on The Therapeutic Effects of Encapsulated Artemisia Annuia and Artemisia Afra

Constant  
Kansango  
Tchandema

MD, Lubumbashi, RDCongo

Pierre Lutgen

PhD, IFBV-BELHERB, Luxembourg

### ABSTRACT

This trial took place in the province of Katanga in the RDCongo and was handled in two parts

- 82 volunteers suffering from malaria were treated during 7 days with capsules containing powdered leaves of *Artemisia annua* from Luxembourg (AAL) or from Burundi (AAB) and *Artemisia afra* (AAF). Total dose for AAL was 15 gr, for AAB 7.5 gr and for AAF 7.5 gr. Despite these low doses all patients were free of fever after 2 days and 85% were free of parasites after 7 days for AAL, 76% for AAB and 40% for AAF.
- 44 volunteers carrying trophozoites were treated with capsules containing *Artemisia afra* leaf powder from Burundi. The total dose of *Artemisia afra* powder administered over 10 days was 20.gr. In order to better understand the prophylactic and therapeutic effect of *Artemisia* herbal medicine on malaria infection it is important to assess the CD4 count and gametocyte carriage before and after treatment. It was found that on day 10 the CD4 count had on the average increased by 20% and the trophozoite carriage was reduced to zero except in a few rare cases

### KEYWORDS :

#### INTRODUCTION

A large portion of the population consults with practitioners of traditional medicine and medicinal uses of local plants for first aid, as only choice in the absence of other remedies (Kitua a.y Malebo & HM 2004). The World Health Organization (WHO) is trying to promote traditional medicine and its integration into medical treatments (WHO Perspectives of Traditional Medicine 2014-2023). *Artemisia annua* is a major source of artemisinin, which has received considerable attention as a promising and powerful malaria drug and its rather low toxicity (Ferreira et al 1995). This active ingredient has been extracted from the plant which has been used in China to treat fever and malaria for centuries (Allahdin O et al. 2008). However, it appears that the antimalarial potential of *Artemisia annua* is not only due to artemisinin; several studies have shown that many other molecules or inorganic constituents in the plant also have their own or synergistic antimalarial activity, and it seems that the importance given to artemisinin has been overestimated (Ferreira et al 2010 ; Fouda , 2010 ; De Donno et al 2012).

In this study the main objective was to compare the therapeutic effects of *Artemisia annua* powder from two different origins to *Artemisia afra* based on clinical and biological signs.

In a second part of the trial the objective was to verify the mode of action of *Artemisia afra*, its impact on the immune system and other physiological factors and confirm the absence of side effects.

#### 1.0 MATERIALS AND METHODS

##### POPULATION STUDY

The study was conducted in the Democratic Republic of Congo in the Katanga Province during a period of 8 months, from April 2013 to December 2013.

The first part of the study population included 82 young adult volunteers with uncomplicated *Plasmodium falciparum* malaria who were divided into three groups:

The first group of 20 volunteers received *Artemisia annua* from Luxembourg

The second group of 37 volunteers received *Artemisia annua* from Burundi

The third group of 25 volunteers received *Artemisia afra*

44 patients were treated with capsules containing *Artemisia afra* leaf powder from Burundi

#### CRITERIA FOR INCLUSION

Any single voluntary adult patient with age varying between 15 and 70 years and with non- complicated form of malaria with *Plasmodium falciparum* confirmed by a positive blood smear

#### EXCLUSION CRITERIA

Pregnant women, children, sick patients under other antimalarial or antibiotics two weeks before treatment.

#### TREATMENT

Three varieties of *Artemisia* were used :

*Artemisia annua* from Luxembourg (low concentration of artemisinin, capsules 1 gram) *Artemisia annua* from Burundi (artemisinin in high concentration, capsules 0.5 gram)

*Artemisia afra* (from Burundi without artemisinin, capsules 0.5 gr)

Each patient received 15 capsules : 3 capsules on the first day and 2 capsules on the six following days, corresponding to a total of 15 grams of *Artemisia annua* from Luxembourg, 7,5 gr of *Artemisia annua* from Burundi, 7,5 grams of *Artemisia afra*.

Paracetamol was prescribed on the first day for patients with fever.

#### RESULTS PART 1

##### 2.1 EPIDEMIOLOGY PROFILE

- The 82 patients treated with capsules of *Artemisia* were divided into three sub -groups:
- For the first group (20 volunteers) who received *Artemisia annua* Luxembourg, 85 % of patients healed at the 7<sup>th</sup> day and clinical signs improved in 100 % of cases within the first two days of treatment.
- For the second group (37 volunteers) who received *Artemisia annua* of Burundi, 75.6 % of patients healed at the 7<sup>th</sup> day and clinical signs improved in 100 % of cases within the first two

days of treatment.

- For the third group (25 volunteers) who received *Artemisia afra* of Burundi, 40% of patients healed at the 7<sup>th</sup> day and clinical signs improved in 100 % of cases within the first two days of treatment.

**2.2 CLINICAL PROFILE**

2.2.1 Group of patients given capsules of *Artemisia annua* Luxembourg (AAL)

**TABLE 1: Distribution of patients by sex, age, clinical signs, and biological evolution**

		Patients (n 20)	%
<b>Gender</b>	male	14	70
	female	6	30
<b>Age</b>	18 – 38	14	70
	39 – 49	6	30
<b>Clinical indications Day 1</b>	Feverish	3	15
	Afebrile	17	85
<b>Clinical indications Day 7</b>	Feverish	0	0
	Afebrile	0	0
<b>Thick film blood smear Day1</b>	+	7	35
	++	3	15
	+++	10	50
<b>Day 7</b>	-	17	85
	+	3	15
	++	0	0
<b>General disease progression</b>	Good progress without side effects	20	100
	Side effects	0	0

**COMMENTS :**

- The sex ratio is 70% of males
- The age between 18 and 38 years included 70% of cases
- 15 % of patients were febrile on the first day and 85% were afebrile.
- At J+10 a complete disappearance of clinical manifestations was observed.
- At J+1 blood smear was positive (+++) in 50 % of patients; at J+7 blood smear was positive in 15% of patients.
- No adverse events were observed on the 25 cases followed

2.2.2 Group of patients given capsules of *Artemisia annua* Burundi (AAB)

**TABLE 2: Distribution of patients by sex , age, clinical signs, and biological evolution**

		Patients (n 37)	%
<b>Gender</b>	male	15	40.54
	female	22	59.45
<b>Age</b>	15 – 25	6	16.21
	26- 36	15	40,54
	37-47	16	43.24
<b>Clinical indications Day 1</b>	Feverish	12	32.43
	Afebrile	25	67.56
<b>Clinical indications Day 7</b>	Feverish	0	0
	Afebrile	0	0
<b>Thick film blood smear Day1</b>	+	23	62.16
	++	12	32.43
	+++	2	5.40

<b>Day 7</b>	-	28	75.67
	+	9	24.32
	++	0	0
<b>General disease progression</b>	Good progress without side effects	37	100
	Side effects	0	0

**COMMENTS :**

- The sex ratio is 61% of males
- The age group between 37 and 47 years included 43% of cases
- 32 % of patients were febrile on the first day and 68% were afebrile.
- At J+10 a complete disappearance of clinical manifestations was observed in all cases.
- At J+1 blood smear was positive in 62 % of patients; at J+7 blood smear was positive in 24% of patients.

2.2.3. Group given capsules of *Artemisia afra* Burundi (AAF)

**TABLE 3: Distribution of patients by sex, age, clinical signs, and biological evolution**

		Patients (n 25)	%
<b>Gender</b>	male	9	36
	female	16	64
<b>Age</b>	18 – 28	6	24
	29 – 39	15	60
	40-49	4	16
<b>Clinical indications Day 1</b>	Feverish	19	76
	Afebrile	6	24
<b>Clinical indications Day 7</b>	Feverish	0	0
	Afebrile	0	0
<b>Thick film blood smear Day1</b>	+	1	4
	++	5	20
	+++	19	76
<b>Day 7</b>	-	10	40
	+	0	0
	++	15	60
<b>General disease progression</b>	Good progress without side effects	25	100
	Side effects	0	0

**Comments :**

- The sex ratio is 36% of males
- The age group between 29 and 39 years included 60% of cases
- 76 % of patients were febrile on the first day and 24% were afebrile.
- At J+10 a complete disappearance of clinical manifestations was observed.
- At J+1 blood smear was positive (+++) in 76 % of patients; at J+7 blood smear was positive (++) in 60% of patients.
- No adverse events were observed on the 25 cases followed

**3.0 RESULTS PART 2**

In the second part of the trial with *Artemisia afra* capsules an average increase of CD4 by 10 % to 20% was noticed and trophozoites disappeared at J+11, with a few exceptions.

In all patients changes in urea, creatinin, ALAT and ASAT were monitored. No significant changes were noticed.

It is difficult to summarize these results in one table. The individual data can be found in the TABLE I in the annex.

## DISCUSSION

The results obtained during this preliminary comparative study between two families of *Artemisia* (*Artemisia annua* and *Artemisia afra*) demonstrate the therapeutic effect of the powdered leaves. It is remarkable that these results were obtained with very low total doses: 15 gr for *Artemisia annua* from Luxembourg, and 7.5 gr for *Artemisia annua* from Burundi and 7.5 gr for *Artemisia afra*. The effects are related to the disappearance of clinical and biological signs; a good evolution is evident in all patients. The efficacy of herbal tea or powder has already been demonstrated in clinical trials in Africa (Rosine Chougouo et al, 2009. Patrick Engeu et al 2011). Our results confirm the results obtained with the *Artemisia annua* powder by Michel Onimus *in vivo*. These results also corroborate those obtained by other medical teams (Saint- Hillier, Klages ) in Africa working with capsules of *Artemisia annua* and *Artemisia afra* powder in adults and children with excellent results. High concentration in artemisinin does not appear to be a key factor. The results obtained with *Artemisia afra* confirm the antimalarial effects of other *Artemisia* species containing no artemisinin, as *Artemisia apiacea* in China, *Artemisia ludoviciana* in California and *Artemisia sieberi* in Iran.

The most likely hypothesis for this antimalarial activity of *Artemisia* plants is their strong hemozoin (beta-hematin) crystallization inhibition (M Akkawi, 2012). This had been recognized in the past as the main mode of action of quinine and chloroquine.

## CONCLUSION

The present study demonstrates that the administration of *Artemisia annua* and *Artemisia afra* powder capsules is an effective way to fight malaria in Africa. The therapeutic efficacy is not dependent on high concentrations of artemisinin contained in the plant and highlights the potentiating effects of other components present in the plant. Our results confirm similar reports on the significant antimalarial efficiency of other varieties of *Artemisia* like *Artemisia afra* without artemisinin and this may contribute to the fight against malaria in Africa.

Our present work, as well as the clinical trials carried out in Maniema in 2015 by Dr Jerome Munya and Michel Idumbo (personal communication) show a complete disappearance of parasites and gametocytes on day 10 and further on. A breakthrough finding which could stop transmission from humans to mosquitoes and fulfill the dream of malaria eradication.

*Artemisia afra* is growing wild in all countries of Eastern Africa from The Cape to Addis Ababa, from Nairobi to Kinshasa. In all these countries it has been widely used and continues to be used against malaria and many other diseases like tuberculosis or bilharzia. It fulfills the requirements of the "WHO Perspectives for Traditional Medicine 2014-2023". It has also shown strong anti-HIV properties (Fr Van der Kooy 2011)

*Artemisia afra* readily accessible to low income populations could become the flagship in the fight against malaria in Africa.

## FUTURE WORK

- Evaluate more precisely the effect of the initial parasite load on the results. In the present work the initial parasite load was significantly higher for AAL and AAF groups than for the AAB group. It has been shown that if the initial parasitic load exceeds 10 000/ $\mu$ l 10x recrudescence can be higher (Ittarat W).

- Monitor the CD4 for different treatments.

- Check if other drugs like aspirin, paracetamol, antiretrovirals, vitamin C and E, iron supplements might explain the exceptional increase in trophozoite carriage at the end of the treatment for a few patients and treatment failure as often observed for ACTs.

WHO Perspectives Traditional Medicine 2014-2023. Dec 2013

## Annex 1. BILAN BIOLOGIQUE DES PATIENTS SOUS ARTEMISIA AFRA TRIMESTRE 1/2014

### PATIENT 1

#### BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	768		920
UREE	27		24
CREATININE	0,9		0,86
ACIDE URIQUE			
GE	10 à 100 trophozoites		1 à 10 trophozoites

### PATIENT 2

#### BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1131		1295
UREE	29		25
CREATININE	0,95		0,89
ACIDE URIQUE			
GE	10 à 100 trophozoites		1 à 10 trophozoites

### PATIENT 3

#### BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1174		1265
UREE	36		28
CREATININE	1,00		0,82
ACIDE URIQUE			
GE	10 à 100 trophozoites		0 trophozoites

### PATIENT 4

#### BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1130		1210
UREE	31		19
CREATININE	0,96		0,90
ACIDE URIQUE			
GE	10 à 100 trophozoites		0 trophozoites

### PATIENT 5

#### BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	655		798
UREE	11		15
CREATININE	0,88		0,84
ACIDE URIQUE			
GE	10 à 100 trophozoites		0

### PATIENT 6

#### BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	850		987
UREE	27		23
CREATININE	0,83		0,86
ACIDE URIQUE			
GE	10 à 100 trophozoites		100 à 1000 trophozoites

### PATIENT 7

#### BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1288		1360

UREE	26		24
CREATININE	0,81		0,9
ACIDE URIQUE			
GE	1 à 10 trophozoites		0 trophozoites

**PATIENT 8**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	894		1020
UREE	25		26
CREATININE	0,8		0,81
ACIDE URIQUE			
GE	10 à 100 trophozoites		0 trophozoites

**PATIENT 9**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	788		930
UREE	29		23
CREATININE	0,91		0,88
ACIDE URIQUE			
GE	1 à 10 trophozoites		0 trophozoites

**PATIENT 10**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1251		1430
UREE	15		17
CREATININE	0,7		0,8
ACIDE URIQUE			
GE	1 à 10 trophozoites		0 trophozoites

**PATIENT 11**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1211		1295
UREE	24		25
CREATININE	0,83		0,8
ACIDE URIQUE			
GE	1 à 10 trophozoites		0 trophozoites

**PATIENT 12**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	890		1110
UREE	28		25
CREATININE	0,92		0,87
ACIDE URIQUE			
GE	1 à 10 trophozoites		0 trophozoites

**PATIENT 13**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1120		1340
UREE	26		21
CREATININE	0,87		0,89
ACIDE URIQUE			
GE	1 à 10 trophozoites		0 trophozoites

**PATIENT 14**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	986		1010
UREE	22		20
CREATININE	0,89		0,86
ACIDE URIQUE			
GE	1 à 10 trophozoites		1 à 10 trophozoites

**PATIENT 15**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1050		1175
UREE	31		26
CREATININE	0,79		0,80
ACIDE URIQUE			
GE	1 à 10 trophozoites		0 trophozoites

**PATIENT 16**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1342		1390
UREE	27		21
CREATININE	1,0		0,9
ACIDE URIQUE			
GE	10 à 100 trophozoites		1 à 10 trophozoites

**PATIENT 17**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1310		1380
UREE	18		20
CREATININE	0,76		0,7
ACIDE URIQUE			
GE	10 à 100 trophozoites		1 à 10 trophozoites

**PATIENT 18**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1248		1300
UREE	21		19
CREATININE	0,81		0,82
ACIDE URIQUE			
GE	10 à 100 trophozoites		0 trophozoites

**PATIENT 19**  
BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1292		1360
UREE	17		15
CREATININE	0,75		0,76
ACIDE URIQUE			
GE	1 à 10 trophozoites		0 trophozoites

**PATIENT 20**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1120		1214
UREE	21		20
CREATININE	0,8		0,82
ACIDE URIQUE			
GE	10 à 100 trophozoites		1 à 10 trophozoites

BILAN BIOLOGIQUE DES PATIENTS SOUS ARTEMISIA AFRA TRIMESTRE 2/2014

**PATIENT 21**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1254		1410
UREE	17		18
CREATININE	0.72		0.73
ACIDE URIQUE			
GE	10 à 100 trophozoites		0 trophozoites

**PATIENT 22**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	756		1010
UREE	29		21
CREATININE	0.87		0.80
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 23**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	810		1098
UREE	30		26
CREATININE	0.91		0.87
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 24**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1231		1379
UREE	16		16
CREATININE	0.65		0.69
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 25**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1174		1282
UREE	17		16
CREATININE	0.74		0.7
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 26**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	817		1191
UREE	26		20
CREATININE	0.9		0.87
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 27**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	637		796
UREE	14		15
CREATININE	0.96		0.89
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 28**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1015		1930
UREE	19		16
CREATININE	0.96		0.6
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 29**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1015		1230
UREE	13		13
CREATININE	0.57		0.6
ACIDE URIQUE			
GE	10 à 100 trophozoites		0 à 10 trophozoites

**PATIENT 30**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	936		1116
UREE	17		15
CREATININE	0.97		0.7
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 31**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1140		1300
UREE	26		18
CREATININE	0.97		0.8
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 32**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1145		1345
UREE	10		13

CREATININE	1.11		0.80
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 33**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	720		980
UREE	11		11
CREATININE	0.86		0.84
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 34**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1232		1310
UREE	20		20
CREATININE	0.93		0.91
ACIDE URIQUE			
GE	10 à 100 trophozoites		0 à 10 trophozoites

**PATIENT 35**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1125		1257
UREE	14		15
CREATININE	0.61		0.6
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 36**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1910		1456
UREE	18		17
CREATININE	0.60		0.64
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 37**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	741		1050
UREE	26		23
CREATININE	0.87		0.85
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 38**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	756		977
UREE	28		25
CREATININE	0.88		0.87
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 39**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1096		1208

UREE	19		20
CREATININE	0.82		0.83
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 à 10 trophozoites

**PATIENT 40**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	816		1081
UREE	22		24
CREATININE	0.8		0.72
ACIDE URIQUE			
GE	10 à 100 trophozoites		0 à 10 trophozoites

**PATIENT 41**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	719		987
UREE	18		18
CREATININE	0.81		0.8
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 42**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	420		758
UREE	17		16
CREATININE	1.1		0.94
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 43**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	849		1108
UREE	19		16
CREATININE	0.63		0.7
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**PATIENT 44**

## BILAN BIOLOGIQUE

Examens para cliniques	J1	Intermédiaires	J11
CD4	1151		1265
UREE	11		14
CREATININE	0.6		0.65
ACIDE URIQUE			
GE	0 à 10 trophozoites		0 trophozoites

**REFERENCES**

1. Akkawi M et al. (2014) Investigations of Artemisia Annua and Artemisia Sieberi Water Extracts Inhibitory Effects on  $\beta$ -Hematin Formation. *Med Aromat Plants*, 3:1.
2. Allahdin O, Gothard-Bassebe MC, Biteman O, Foto E, Mabingui J, et al. (2008). Essai de désinfection de l'eau de puits par l'Artemisia annua. *Revue Technique Luxembourgeoise* 3: 165-168.
3. Chougouo R. & P Lutgen (2010): Physico-chemical characteristics of Artemisia annua antimalarial plant. Laboratoire National de la Santé, Luxembourg.
4. Chougouo R.; Kouamouo J. et al, Université des Montagnes, Cameroun, MIM Pan-African Malaria Conference from 2nd to 6th November 2009. Nairobi-Kenya.
5. Chougouo R, Kouamouo J, Moyou R, Penge A. (2012) Comparative study of the therapeutic efficacy of Artesunate alone or in combination with Amodiaquine and tea of Artemisia annua cultivated in West Cameroon. *J Pharm Biom Nat Sci*;1(4):127-48.
6. Chougouo K. Rosine D. et al. Comparative Hepatoprotective and Antioxidant



- Activities of Artesunate and Flavonoids extracts from *Artemisia annua* grown in Cameroon Submitted for publication.
7. De Donno A et al. (2012) First-time comparison of the in vitro antimalarial activity of *Artemisia annua* herbal tea and artemisinin. *Trans R Soc Trop Med Hyg.* Nov;106(11):696-700. doi: 10.1016/
  8. Fouda E. (2010). Etude clinique sur l'efficacité thérapeutique de l'*Artemisia annua* sur l'accès palustre simple. District de santé de la Cité Verte, Yaoundé, personal communication.
  9. Elfawal MA, Towler MJ, Reich NG, Golenbock D, WeathersPJ, Rich SM et al. (2012) Dried Whole Plant *Artemisia annua* as an Antimalarial Therapy. *PLoS ONE.*7(12):e52746.
  10. Ferreira JFS, Janick J (1995). Production and detection of artemisinin from *Artemisia annua*. *Acta Horticulturae*, 390: 41–49.
  11. Gueye O. Chimiosensibilité ex vivo des souches de *Plasmodium falciparum* à la tisane d'*Artemisia annua* et caractérisation des éléments minéraux contenus dans les feuilles de la plante [Thèse]. Biologie et pathologies humaines: Dakar ; Université Cheikh Anta Diop; 2008.
  12. Hsu E. Reflections on the 'discovery' of the antimalarial qinghao. *Br J Clin Pharmacol.* 2006;61(6): 666–670.
  13. Icipe, Kemri and Nusag. Whole-leaf artemisia annua-based antimalarial drug: report on proof-of-concept studies. Nairobi: International Centre of Insect Physiology and Ecology; 2005
  14. Ittarat W, Pickard A et al., Recrudescence in Artesunate treated patients with falciparum malaria is dependent on parasite burden, *Am J Trop Med Hyg* 68, 2003, 147-152
  15. Kitua AY, Malebo HM. (2004): Malaria control in Africa and the role of traditional medicine. In *Traditional Medicinal Plants and Malaria*. Edited by Willcox ML, Bodeker and Rasoanaivo P. Boca Raton: CRC Press
  16. Kofoed Paul-Erik, Ursing Johan et al., Paracetamol versus placebo in treatment of non severe malaria in children in Guinea-Bissau. *Malaria Journal* 2011, 10:148
  17. Lamero C. Contribution à l'étude chimique des flavonoïdes d'*Artemisia annua* [Thèse]. Pharmacie: Bangangté ; Université des Montagnes ; 2012, 63p.
  18. Lee IS, Kim KS, Jang JM, Park Y, Kim YB, Kim BK. Phytochemical constituents from the herba of *Artemisia apiacea*. *Arch Pharm Res.* 2002;25(3):285-8.
  19. Lehane AM, Saliba K. Common dietary flavonoids inhibit the growth of the intraerythrocytic malaria parasite. *BMC Res Notes.* 2008;1:26.
  20. Lutgen P, Michels B. Bactericidal properties of *Artemisia annua* tea and dosimetry of artemisinin in water by fluorescence under UV light. 2008. *Revue Technique Luxembourgeoise*, 2008, 2 ; p73-78.
  21. Lutgen P; A. Keynote presentation given at the WESA conference Phnom Penh on 3rd Dec 2010 B. Ponencia al Congreso Latinoamericanos de Parasitología Bogota on 29th Oct. 2011. Presentation made at the second Malaria Conference. Copenhagen on 12th April 2012, 13th Internat Congress on parasitology, Aug 2014 Mexico City
  22. Lutgen P. Tisane d'*Artemisia annua*, une puissante polythérapie. Rapport sur le 2ème congrès «Maladies tropicales, aspect humanitaire et scientifique». Luxembourg ; 2009. Malagon F, Vazquez J, Delgando G, Ruiz A. Antimalaric effect of an alcoholic extract of *Artemisia ludoviciana* Mexicana in a rodent malarial model. *Parasitologia.* 1997;39(1):3-7.
  23. Mueller MS, Karhabomga IB, Hirt HM, Wemakor E (2000): The potential of *Artemisia annua* L; As a locally produced remedy for malaria in the tropics: Agricultural, Chemical and Clinical aspects. *J Ethnopharmacol* 73: 487-493.
  24. Ntutela S, et al., Efficacy of *Artemisia afra* phytotherapy in experimental tuberculosis. *Tuberculosis (Edinb).* 2009 Dec;89 Suppl 1:533-40. doi: 10.1016/S1472-9792(09)70009-5.
  25. Ogwang PE, Ogwal J O, Kasasa S, Ejobi F, Kabasa D, et al. (2011): Use of *Artemisia annua* L. Infusion for Malaria Prevention: Mode of Action and Benefits in a Ugandan Community. *British J of Pharm Research* 1: 124-132.
  26. Ogwang PE, Ogwal JO, Kasasa S, Ejobi F, Kabasa D, Obua C. Use of *Artemisia annua* L. Infusion for Malaria Prevention: Mode of Action and Benefits in a Ugandan Community. *British J of Pharm Research*, 2011;1(4):124-39.
  27. Onimus M, Carteron S, Lutgen P. The surprising efficiency of *Artemisia annua* powder capsules. *Med Aromat Plants.* 2013;2:125.
  28. Räch K, Taxis K, Walz G, Gleiter CH, Li SM, Heide L. Pharmacokinetic study of artemisinin after oral intake of a traditional preparation of *Artemisia annua* L. *Am J Trop Med Hyg.* 2004;70(2):128-32.
  29. Sunmonu T and Afolayan A, Evaluation of Antidiabetic Activity and Associated Toxicity of *Artemisia afra* Aqueous Extract in Wistar Rats. *Evidence-Based Complementary and Alternative Medicine* Volume 2013 (2013), Article ID 929074.
  30. Tiruneh G, Kebede Y, Yigzaw T. Use of the plant *Artemisia annua* as a natural anti-malarial herb in Arbaminch town. *Ethiop J Health Biomed Sci.* 2010;2:76-82.
  31. Van der Kooy F *Reverse Pharmacology and Drug Discovery: Artemisia and Its Anti-HIV Activity*. Aftab et al. (eds.), *Artemisia annua - Pharmacology and Biotechnology*, DOI: 10.1007/978-3-642-41027-7\_14, Springer-Verlag Berlin Heidelberg 20
  32. Weathers P; Arsenault P; Covello P; McMickle A; Teoh K; et al. (2011): Artemisinin production in *Artemisia annua*-studies in planta and a novel delivery method for treating malaria and other neglected diseases. *Phytochem Rev* 10: 173-183.
  33. Weathers PJ, Arsenault PR, Covello PS, Mickle A, Tesh KH, Reed DW. Artemisinin production in *Artemisia annua*: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases. *Phytochem Rev.* 2011;10(2):173–83.
  34. Weathers PJ, Jordan NJ, Lasin P, Towler MJ. Simulated digestion of dried leaves of *Artemisia annua* consumed as a treatment (pACT) for malaria. *J Ethnopharmacol.* 2014;151:858-63.
  35. Weathers PJ, Towler MJ. Changes in key constituents of clonally propagated *Artemisia annua* L. during preparation of compressed leaf tablets for possible therapeutic use. *Ind Crop Prod.* 2014;62:173-78.
  36. Willcox ML, Burton S, Oyweka R, Namyalo R, Challand S, Lindsey K. Evaluation and pharmacovigilance of projects promoting cultivation and local use of *Artemisia annua* for malaria. *Malaria J.* 2011;10:84..