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Activity of Artemisia Annua Capsules Versus Artemeter Lumefantrine On Uncomplicated Malaria.

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ABSTRACT
The effectiveness of Artemisia annua herbal tea on uncomplicated malaria has been demonstrated by many studies. However, the herbal tea remains a rather restrictive galenic form. The present study aimed to develop Artemisia annua leaf capsules and to compare their anti-malarial activity with that of the artemeter-lumefantrine combination currently widely used in the treatment of malaria.
The juice of the leaves of Artemisia annua was dried in an oven at 45°C and then the powder was put into capsules according to the conventional capsule manufacturing protocol. After a physicochemical and pharmaco-technical control, the artemisinin content was determined by TLC-densitometry and the flavonoid content by spectrophotometry. The capsules obtained were administered in parallel with artemeter-lumefantrine tablets to a global sample of 149 patients (63 for artemeter-lumefantrine and 86 for Artemisia annua), in search of anti-malarial activity and possible side effects.
Artemisia annua powder was obtained with a yield of 13.90%; it contained 1.23% artemisinin and 51.2 mg quercetin equivalent (QE) of total flavonoids. The formulated capsules (Palu Stop * capsules) were size n°2 and contained 250mg of powder corresponding to 3mg of artemisinin and 12.8mg QE of total flavonoids. Against malaria they were administered at a daily dose of 2 capsules twice for adults and 1 capsule twice for children. The artemether-lumefantrine combination and the Artemisia annua capsules (PaluStop *) regressed the clinical signs very early from the second day of treatment. Both products lowered the temperature in 3 stages with a return to normal before the 7th day of treatment; in addition, they caused a rapid decrease in parasitemia in the first 3 days of treatment. In terms of clinical response, no early failure was observed; however, with artemether-lumefantrine a late failure rate of 6.35% was recorded in contrast to Artemisia annua. Blood pressure, blood sugar and creatinine levels were not affected by either product; however, both caused a small transient rise in transaminases. As regards side effects, a slight pruritus was observed on the first day of treatment with Artemisia annua in a few patients (1.16%); this pruritus subsided the next day in the presence of chlorpheniramin.
Artemisia annua capsules (PaluStop* capsules) have shown the same therapeutic interest as the artemeter-lumefantrine combination.

KEY WORDS:, anti-malarial, Artemisia annua, artemeter-lumefantrine, capsule, efficacy, tolerance.

I. INTRODUCTION

Malaria is one of the greatest scourges still threatening humanity. Africa is the continent most affected by this disease. In 2018 it was estimated that 10,000 pregnant women and 20,000 children died of malaria [1]. Today WHO recommends Artemisinin-based Combination Therapy (ACT) for the treatment of uncomplicated malaria [2,3], yet numerous studies have shown that Artemisia annua leaf tea is effective against malaria [4,5,6,7]. Other studies have shown that treatment with Artemisia annua is not a monotherapy because of its composition but a true combination therapy [8,9,10]. The constraints of preparation of the herbal tea, the instability of artemisinin during a long stay in an aqueous medium [6] and its stability in a dry environment [11], motivated us in the present study to try to formulate an improved traditional medicine (ITM) in capsule form and to evaluate its efficacy compared to the artemeter-lumefantrine combination.

II. MATERIAL AND METHOD

Material

The equipment used included an electric grinder, an oven, chromatography plates, a capsule jar, culture media, Coartem* (artemeter lumefantrine) tablets and a technical data sheet.

Preparation of the ITM in capsule form

The fresh leaves of Artemisia annua were cleaned with purified water and then crushed. The resulting porridge was pressed in a sterile double compress, filtered and dried in an oven at 45°C. The dry residue obtained was ground in a mortar and then subjected to physico-chemical and rheological controls [12,13,14]. On the basis of the quantity of artemisinin contained in the daily dose of herbal tea [15], the daily quantity of powder was determined and used to fill the capsules with simple and easily available excipients [16,17].
The capsules obtained were subjected to the usual physicochemical, pharmacotechnical and microbiological controls [1,17,18].

Research of the effectiveness and tolerance of the capsules in comparison with coartem * tablets (artemeter-lumefantrine combination)

Administrative formalities:

The study took place in the locality of LOKOTI in the region of Adamaoua on a population of 149 patients (86 for Artemisia annua and 63 for artemeter-lumefantrine).
The study required obtaining ethical clearance from the Institutional Ethics Committee of the Université des Montagnes N°187 of 11/04/2019, a research authorisation from the director of the LOKOTI Integrated Health Centre, the signature of an informed consent form by each patient or the parents for the children.

Study population.

The target population was the population of the Adamaoua region; the source population was the patients of the LOKOTI Integrated Health Centre. As inclusion criteria, we selected patients suffering from uncomplicated malaria, aged over 7 years, with a temperature between 37.5°C and 39.5°C and willing to sign (or obtained the signature of a parent in the case of children) the informed consent form. The patients selected were not to be suffering from severe malnutrition; their infestation was to be confirmed by a thick drop. Given the intensity of transmission in the Adamaoua region, their initial parasite density should be between 1000 and 7000 trophozoites/mm^3. As exclusion criteria, we retained: patient indiscipline and withdrawal of consent.

Administration of the drug.

Patients meeting the inclusion criteria were divided into 2 groups (A and B). The 86 patients in group A received Artemisia annua capsules according to the determined dosage for 7 days while the 63 patients in group B received Coartem* tablets according to their weight for 3 days as recommended by the manufacturer.
Follow-up and monitoring of treatment:
We maintained contact with patients at least every other day to ensure discipline in taking the treatment and to record everyone's opinion on the effectiveness and tolerance of the treatment. The evolution of some of the patients' biological parameters was noted: ASAT, ALAT, Creatinin, CRP, glycaemia [19].
Patients were followed from Day0 to Day28 with recording of thermal clearance, parasite clearance, clinical side effects, abnormal biological examination results. The individual parasitaemia was evaluated by the formula [20] $P = \frac{Np.800}{200}$

With P = parasitemia in number of trophozoites / mm^3 of blood ;

Np = number of parasitized red blood cells equivalent to 200 trophozoites read simultaneously.

III. RESULTS

Powder quality control

The powder obtained with a yield of 13.9% was crumbly and greenish in colour. Its residual moisture content was 9.7 % ± 0.25 %. Exposed to laboratory conditions (25°C temperature and 71% relative humidity) the powder absorbed 2% moisture after 30 minutes. This absorption stabilised at 2.5% after 30 minutes.
In the sieve analysis by the sieve method 51.24 % were found in the 125 µm sieve, the median particle size (D50) was between 125 and 300 µm.

TLC-Densitometry with migration into the n-hexane-diethyl ether mixture (24:20), revelation with acetic acid-sulphuric acid-anisaldehyde mixture (200:4:2) and reading with Mesurim (figures 1 and 2) established that the artemisinin content of the powder was 1.23%.

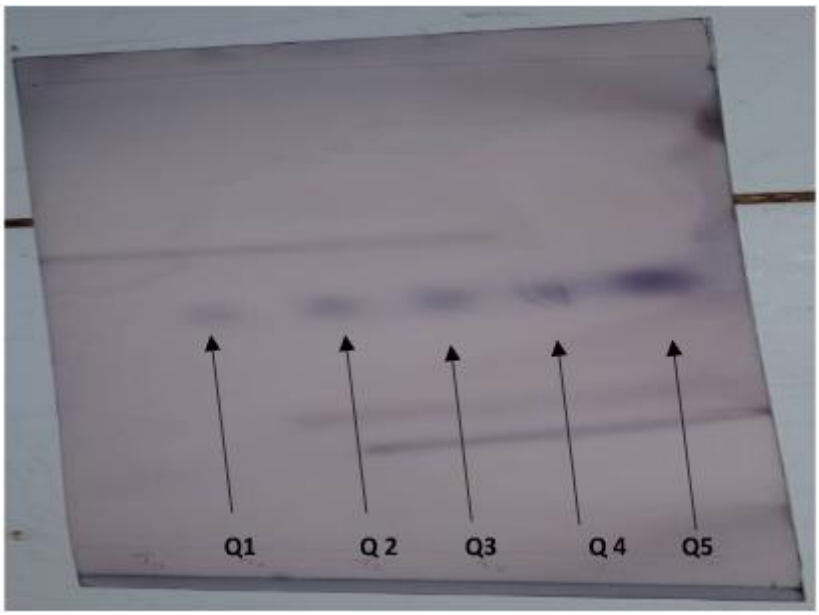

Figure 1: TLC of the calibration range

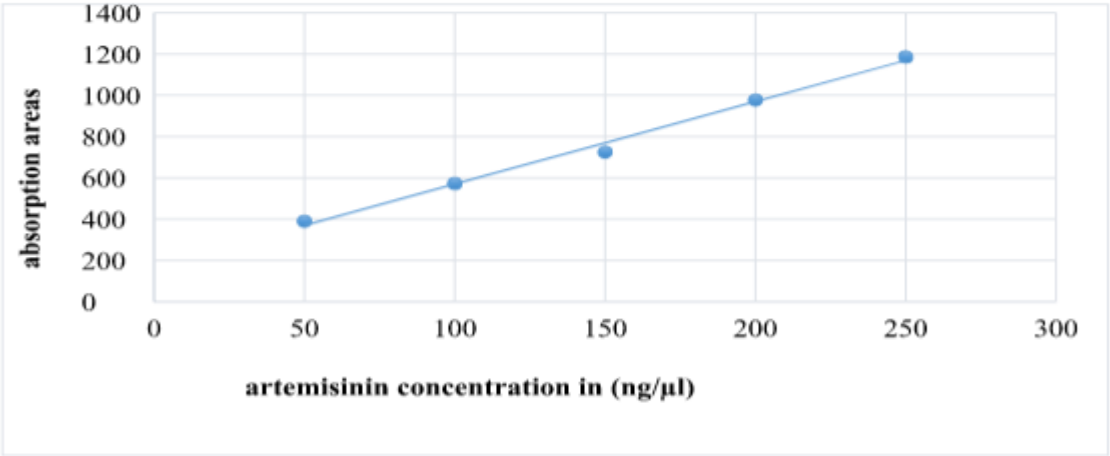

Figure 2: Calibration line

The flavonoid content determined by spectrometry at 415 µm was 51.2 mg quercetin equivalent (QE).

Capsule formulation and control

Daily dose.

The effective daily dose of artemisinin determined by previous studies [4,7] in herbal tea was 12 mg for an adult. This corresponds to 976 mg of our powder, considering the 1.23% content found. We have rounded up this daily dose to 1000 mg.

The pharmacotechnical characteristics of the powder are gathered in table 1.

Table 1. Pharmacotechnical characteristics of the powder

Settings	Results	Comments
Fluidity	4,88 s	Lubricant = silica at a rate of 1,5 %
Bulk density	0,68 g/ml	
Volume without compaction = V0	100 ml	
Volume after 10 settlements = V10	93 ± 0,58 ml	
Volume after 500 settlement = V500	87 ± 0,1 ml	
Volume after 1250 settlements = V1250	72,25 ± 04 ml	
Hausner Index	1,17	Good Floability
Carr Index	13 %	Good Compressibility
V10 – V500	6,5	Good Reorganisation

Capsule formulation and filling

The capsule filling formula shown in Table 2 is compatible with capsule N° 2 corresponding to 2 capsules twice a day for an adult and 1 capsule twice a day for a child.

The name **PaluStop*** has been adopted for these capsules. Filling was carried out at bench scale in batches of 100 capsules on a semi-automatic capsule filler.

Table 2: Unit formula of Palu Stop* capsules

	Quantity	Role
Artémisia annua powder	250 mg	Active principle
Colloïdal Silica	3,75 mg	Lubricant
Wheat starch	30 mg	Thinner

Control of Capsules

The capsules obtained (Figure 3), containing 3 mg of artemisinin and 12.8 mg QE of total flavonoids, were packaged in packs of 28 in hermetically sealed pvc boxes and in the presence of a desiccant capsule. The regulatory controls have given the results gathered in tables III and IV from which it appears that the capsules obtained comply with the standards of the European Pharmacopoeia.

Figure 3: PaluStop* capsules

Table III: Pharmacotechnical controls of PaluStop* capsules

Organoleptic characteristics	Clean, smooth, glossy, dent free, ivory coloured capsules, size 2
Mass uniformity	Average mass = 287.9 mg Tolerable limit deviation e =10 % = 28.79mg Terminals : 259.11 mg and 316.69 mg. None of the 20 unit masses were out of bounds.
Disintegration time	6 minutes at 37° C
Moisture content	At D_0 : 11.35 ± 0,01 % At D_{30} : 11.37 ± 0,01 %
Artemisinin content	At D_0 : 3.05 ± 0.05 mg At D_{30} : 3.05 ± 0.05 mg

Table IV: Results of the microbiological control of PaluStop* capsules

Germ	Media	Temperature	Results
Total germs	Soy tripticase	35° C	No cultivation
Escherichica coli	Mac Conkey	35° C	No cultivation
Staphylococcus aureus	Vogel - Johnson	24 hours at 37° C	No cultivation
Pseudomonas aéruginosa	Cetrimide	24 hours at 37° C	No cultivation

Therapeutic efficacy and tolerance of the 2 drugs.

Parameters related to the study population.

Of the 196 patients received, 27 dropped out, 20 were not selected and 149 were retained.

The parameters related to the study population are grouped in Table V.

Table V: Parameters related to the study population

		Artemeter lumefantrine	Artemicia annua	P value	Staff	Percentage
Sex	Male	32	42	0.093		
	Female	31	44			
Age	[7 to 9[	13	19	0.010		
	[9 to 10[	17	17			
	[10 to 20[	14	20			
	[20 to 60[	11	19			
	[60 to 80[	8	11			
Profession	Dealers				29	19.46
	Cultivator				18	12.08
	Breeder				19	12.75
	Student				65	43.62
	Unemployed				18	12.08
Educational level	None				49	32.89
	Primary				65	43.62
	Secondary				35	23,49
Religion	Muslim				92	61.74
	Christian				57	38.26

The patients selected were of both sexes, ranging in age from 7 to 80 years, with the most represented age group being young people between 7 and 20 years of age. The patients were active in several fields, but the majority were primary school pupils (43.62%).

Evolution of clinical signs.
Table VI shows that for both Artemisia annua (PaluStop*) and artemeter- lumefantrine, the clinical signs regressed early from the 2nd day of the medication and disappeared before the 7th day.

Table VI: Evolution of clinical signs in patients on AL and AA (in %)

	D_0		D_2		D_3		D_7		D_{14}		D_{28}	
	AL	AA	AL	AA	AL	AA	AL	AA	AL	AA	AL	AA
Asthenia	64	84	10	14	5	0	0	0	0	0	0	0
Headake	53	60	10	16	7	3	0	0	0	0	0	0
Myalgia	42	53	20	8	0	2	0	0	0	0	5,2	0
Shivers	46	49	13	12	0	6	0	0	0	0	0	0
Digestive disorders	30	39	27	11	0	0	0	0	0	0	0	0
Vertigo	49	43	4,53	10	2	0	0	0	0	0	0	0
Hyperthermia	32	28	13	17	8	4	6	2	0	0	0	0
Anorexia	48	32	19,6	0	0	0	0	0	0	0	0	0

AL = Artemeter lumefantrine AA = Artemisia annua
The figures represent the percentage of patients with the signs

Evolution of the average temperature of patients under treatment.
At the beginning of the treatment all patients had a temperature > 39° C; with each of the two drugs there was only a three-step drop in temperature with a return to normal at around Day14.

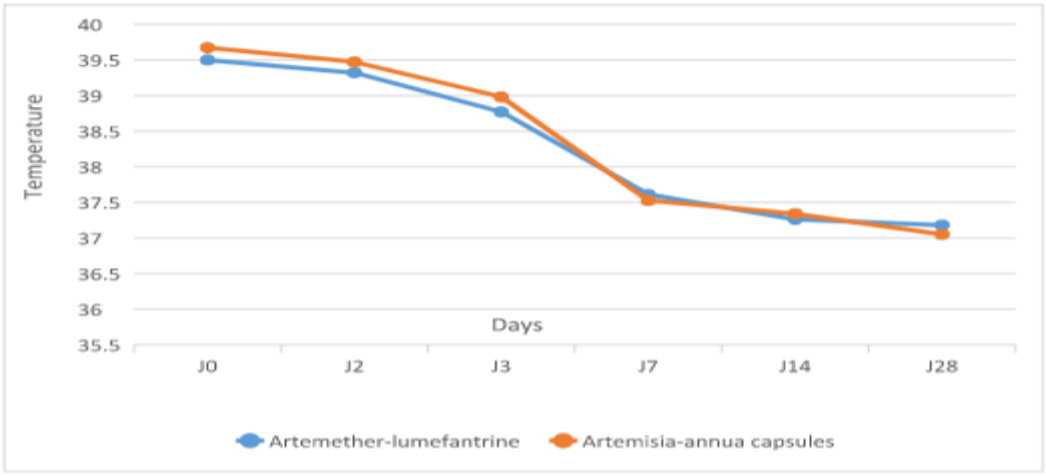

Figure 4: Mean temperature curve of treated patients

Evolution of parasitaemia

In the presence of the two drugs, parasitaemia regressed showing two stages: a fast stage between Day 0 and Day 3 and a slow stage after Day 3.

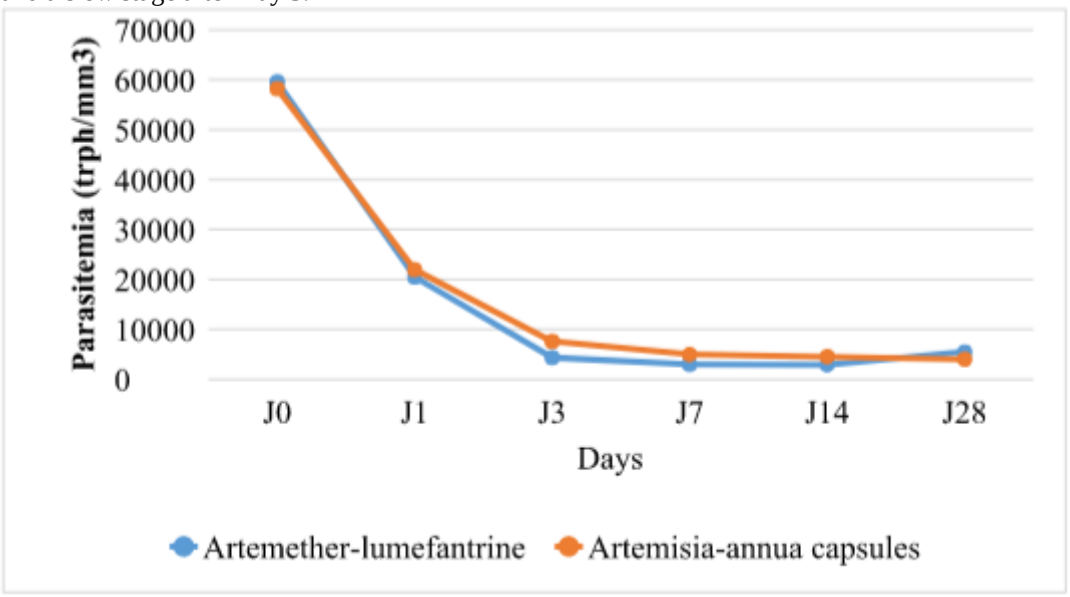

Figure 5: Curve of Parasitemia evolution during treatment

Clinical response

No early failures were observed with either drug. However, late failure was observed in 6.35% of patients treated with artemeter lumefantrine. Therapeutic success is therefore estimated at 100% with Artemisia annua and 93.65% with artemeter-lumefantrine.

Clinical responses	Artéméther-Luméfantrine	Artemisia-annua capsules
Early therapeutic failure	0	0
Late therapeutic failure	4 (or 6.35%)	0
Therapeutic Success	59 (or 93.65%)	86 (i.e. 100%)

Table VII: Clinical response

EARLY THERAPEUTIC FAILURE: subjects presenting at Day3 a persistent fever and a parasitemia with a density greater than 25% of the parasitemia at Day 0.

LATE THERAPEUTIC FAILURE: subjects presenting at Day 3 a parasite density lower than 25% of Day 0 but having presented a fever with a parasitemia between Day 14 and Day 28.

THERAPEUTIC SUCCESS: subjects without fever on Day 3 and with a parasitemia less than 25% of Day 0 and whose parasitemia disappeared between Day 14 and Day 28.

Evaluation of patient tolerance to both treatments.

Biological parameters

Table VIII: Biological control tests.

	Artémisia Annua capsules				Artéméther-Luméfantrine			
	D_0	**D_7**	**D_{14}**	**D_{28}**	**D_0**	**D_7**	**D_{14}**	**D_{28}**
Creatinine	N : 76 A : 0	N : 75 A : 1	N : 76 A : 0	N : 76 A : 0	N : 73 A : 0	N : 73 A : 0	N : 73 A : 0	N : 73 A : 0
ALAT	N : 73 A : 3	N : 74 A : 2	N : 76 A : 0	N : 76 A : 0	N : 69 A : 4	N : 72 A : 1	N : 73 A : 0	N : 73 A : 0
ASAT	N : 72 A : 4	N : 74 A : 0	N : 74 A : 0	N : 74 A : 0	N : 70 A : 3	N : 73 A : 0	N : 73 A : 0	N : 73 A : 0
Blood glucose	N : 26 A : 0	N : 26 A : 0	N : 26 A : 0	N : 26 A : 0	N : 23 A : 0	N : 23 A : 0	N : 23 A : 0	N : 23 A : 0
HTA	N : 26 A : 0	N : 26 A : 0	N : 26 A : 0	N : 26 A : 0	N : 23 A : 0	N : 23 A : 0	N : 23 A : 0	N : 23 A : 0
CRP	N : 65 A : 11	N : 69 A : 7	N : 76 A : 0	N : 76 A : 0	N : 59 A : 14	N : 67 A : 6	N : 73 A : 0	N : 73 A : 0

- N: normal
- A: abnormal

normal values: -creatinine: 7-13 mg / l
-ALAT: Men: < 45UI / L; Women: < 34UI / L
-ASAT: Men: < 35 IU / L; Women: < 35 IU / L
-Glycemia: 0.7 to 1.10 g / l
-TA < 120/80 mmHg
-CRP: < 6mg / L

Table VIII summarises the evolution of some biological parameters in patients undergoing the 2 treatments: Creatinemia remained stable throughout the study. The same applies to blood sugar levels. With regard to hepatic parameters, the increase in ASAT and ALAT observed between Day 0 and Day 7 disappeared progressively between Day 8 and Day 28. The transitory increase in question did not cause the normal values to be exceeded. Blood pressure monitoring at the various appointments did not reveal any changes that could suggest a cardiovascular disease.

Side effects.

Mild pruritus was observed during the first day of treatment in 1.16% of patients taking Artemisia annua. The pruritus vanished the next day with the use of chlorpheniramin.

No other clinical manifestations were recorded apart from those related to malaria.

IV. DISCUSSION

Capsules are a dry galenic form that can better preserve the active ingredient than herbal tea. The use of the juice of the leaves has made it possible to concentrate the artemisinin sufficiently so that the effective daily dose for adults can be included in 4 capsules administered in 2 doses. This is a big advantage over the 10 capsules needed with the Nkamendjo's preparation [16]. The absence of microorganisms in the capsules confirms the antimicrobial activity of the plant as announced in 2008 by Lutgen [7] and in 2014 by Bilia [21].

The artemisinin dosage by TLC-Densitometry is less accurate than HPLC but gives satisfactory results at a lower cost. The therapeutic efficacy obtained after 7 days for Artemisia annua (100%) is better than that obtained with artemeter-lumefantrine (93.65%). The success of Artemisia annua is due to the fact that, in addition to artemisinin, there are other compounds that potentiate its activity, such as flavonoids, terpens and coumarins [21,22,23]. This 100% therapeutic success had already been reported in 2012 by Chougouo [4] and by Chen Lu in 2018; but in 2010, Fouda announced a success rate of 85.9% [24] for Artemisia annua . The sesquiterpens already highlighted by Elfawal also contribute to the success of Artemisia annua by promoting the absorption of artemisinin [24].

The non-disruption of key biological parameters corroborates Aftab's 2014 results [25].

V. CONCLUSION

At the end of this study, which aimed to develop artemisia annua capsules and compare their activity with that of the artemeter-lumefantrine association, we affirm that the preparation of an ITM in capsules with

the leaves of artemisia annua is relatively simple and leads to a product of good quality, with the same efficacy and tolerance as artemeter-lumefantrine association.

The path thus cleared is promising for the industrial formulation of a new and effective antimalarial drug, alongside those currently in use. Moreover, this galenic form is easy to use, which could encourage research into the effectiveness of Artemisia annua's other claims, particularly its immunostimulant, anti-inflammatory and anti-tumour effects.

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