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The antimalaria role of traditional Chinese drugs *Pasta ulmi* and *Chrysanthemum morifolium ramat* extracts *in vivo* and *in vitro*

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Abstract
It has been found from antimalaria experimental research that *Pasta ulmi* extracts have good effect in both animal malaria models and cell culture. The *Pasta ulmi* is prepared from *Ulmus macrocarpa Hance* and Chrysanthemum, but which of the drugs has given the extracts the antimalaria role is yet to be discovered. Nevertheless, it has been verified from experiments on animals that the antimalaria effect is from the traditional Chinese drug *Chrysanthemum morifolium Ramat* as a component of *Pasta ulmi.* The antimalaria effect of *Chrysanthemum morifolium Ramat* is similar to that of *Pasta ulmi)*and is satisfactorily effective for erythrocytic phase, exoerythrocytic phase, chloroquine sensiible strain, chroloquine resistant strain of malarial parasite, as well as P. falciparum. Hence the understanding that the antimalaria effect of *Pasta ulmi* is from *Chrysanthemum morifolium Ramat.*

Keywords: *Chrysanthemum morifolium Ramat,* malaria research, antimalaria drugs.

1. Introduction
After winning a scholarship from Alexander von Humboldt Stiftung, the author went to Germany to engage in antiparasitic experimental research using traditional Chinese drugs brought from home. In the course of time, it was discovered that traditional Chinese drug *Pasta ulmi* has antimalaria effect through the use of animal models and cell culture. *Pasta ulmi* is made from *Ulmus macrocarpa Hance* and chrysanthemum, but from where it gets its antimalaria effect remains unknown. Later, after a series of investigations, it was verified that *Pasta ulmi*'s antimalaria effect originated in the traditional Chinese drug *Chrysanthemum morifolium Ramat* from then on, research focus was shifted to this aspect [1].

2. Materials and Methods
2.1 *Pasta ulmi*
Pasta ulmi [1] is extracted using 70% ethanol, the total extract (A) obtained by retrieving ethanol. The total extract (A) is then extracted using 99% methanol and water, the extract obtained from the former is lipophil extract (C), the latter water extract (B). Five chromatographic components are obtained from the lipophil extract (C) via chromatography, namely, C0 (RF=0), C1 (RF=0.60), C2 (RF=0.45), C3 (RF = 0.27, and C4 (RF = 0.15). The extracts A, B, and C are prepared as suspensoid with a concentration of 100mg/ml with 5% cremophor physiological saline [2]. The NMRI- white mice of the therapeutic group are given the above-mentioned suspensoid for intra abdominal injection twice a day at a dosage of 10mg (0.1 ml)/25g (weight). For the control group, the mice are given the same dosage of physiological saline intra abdominal injection. The NMRI-white mice each weighing 25g as experimental animals raised in an animal room at temperature 25 ^{0}C, relative humidity 55~65%, and day/night transfer regulation every 12 hrs. Fed with standard food and tap water. During the artificial cultivation of P. falciparum, the cultivation medium is prepared from RPMI 1640 in accordance with Trager' and Jensen's method by using the *Pasta ulmi* lipophil extract (C)'s chromatographic components C0, C1, C2, C3, and C4, whose concentrations in the cultivation medium are 100 ng/ml, 1 μg/ml, and 10 μg/ml, respective.

2.2 *Chrysanthemum morifolium Ramat*
Chrysanthemum morifolium Ramat [3] is extracted with 70% ethanol retrieved. The extract is extracted once more using chloroform and ethylacetate, respectively. The chloroform extract is prepared as 70mg/ml suspensoid with physiological saline for use by animal models.

That is, young white rats infected with *P. yoelii* are given intra abdominal injection 2 and 24 hrs each after infection, dosage 700 mg/kg. The ethylacetate extract is used for cell cultivation. Add 50 μg, 70 μg, 80 μg, 90 μg, and 100 μg ethylacetate extract, respectively, to the cultivation medium prepared for observation of growth and development of P. falciparum. One control group and five therapeutic groups are set up for this experiment, the temperature for cultivation being 37 ± 0.5 ºC. The sporozoite of *P. yoelii* [4]. Three days after eclosion, the Anopheles sp. raised under standard conditions are made to suck blood of anaesthetized white mice already infected with P. yoelii and still raised in the mosquito room at 24 ± 1 ºC. In 15 ~20 days sporozoites are segregated. For every young white rat's weight of 100 g, 700×10^3 pathogen is injected by vena caudalis. The animals infected with malarial parasites are divided into the control group and therapeutic groups, each made up of 10 animals. Histological examination of the liver: Forty hours after an infection of P. yoelii the young white rats are executed, their livers taken out to determine their volumes. And a slice of liver tissue is taken from the left lobe of the liver. This tissue is treated as a frequently used pathological specimen, stained with haematoxylin-eosin, the area of the section no less than 800 mm^2. Calculate the percentage of the liver cells infected with malarial parasites, the diameter of the histotype schizont, and the maturity of histotype schizont. The P. falciparum is provided by Hainan Provincial Institute of Tropical Diseases and prepared as cultivation medium using RPMI-1640 (Gibco) in accordance with Trager' and Jensen's method. The cultivation medium is replaced once every 24 hrs and Giemsa (Sigma USA) is used as the blood stained film. In order to calculate the infectiosity of malarial parasites, it is necessary to calculate over 1000 erythrocytes at a time.

3. Results

For the white mice in the therapeutuic group that received *Pasta ulmi* total extract (A) [3], comparison of their parasitemia with that of the control group shows that the former is slightly arrested. When the animals in the control group all died on the fifth day, all animals of the therapeutic group were still alive. No antimalaria effect has been seen in the *Pasta ulmi's* water extract. On the contrary, the *Pasta ulmi* lipophil extract (C) exhibits good antimalaria effect in the anti-P. Vinckei experiment. The parasitemia with this therapeutic group completely vanished on the fifth day and the experimental animals all survived. Therapeutical effect observation is made of the artificially cultivated P. falciparum using the *Pasta ulmi* lipophil extract's chromatographic components C0 and C3. When the concentration of C0 and C3 in the cultivation medium is raised to 10μg/ml, not only is the parasitemia in the said two therapeutic groups developing slowly, but also it is weak in strength compared with the control group. On the fifth day, then P. falciparum in erythrocytes are all eliminated. The sporozoites injected into the animals'blood quickly made their way into the liver cells. As a rule, they will develop into histoschzonts. Because of the action of *Chrysanthemum morifolium Ramat* chloroform extract, the process of development [4] of exoerythrocytic plasmodia is changed. It is shown by experiment on animals that it's impossible for the *Chrysanthemum morifilium Ramat* chloroform extract to prevent sporozoites getting into the liver cells. But it plays a distinct role in suppressing sporozoites schizogony in the liver cells and development into exoerythrocytic schzontes. It is the first time that such an antimalaria effect of the *Chrysanthemum morifolium Ramat* is ever discovered. The development rates of sporozoites for the therapeutic and control groups, the diameters of the mature histotype schizontes, and the maturities of the histotype schizontes are given in the following table.

Table 1: The suppressing action of *Chrysanthemum morifolium Ramat* chloroform extract on exoerythrocytic phase plasmodia ($\overline{X}\pm S$)

group	Sporozoite deverop-ment rate (%)	average diamer of maturity of histotype	Mature histotype schizonte (%)	Schizontes (μm)
Therapeutic group	4.09±5.49		19.55 ±3.79	6.66
control group	10.08±4.92		31.39±5.98	88.33

No appreciable difference (P>0.05) has been observed in the liver cell sporozoites infectiosity between the animals in the therapeutic group and those in the control group after treatment with the *Chrysanthemum morifolium Ramat* chloroform extract. The diameter of the therapeutical group animals histotype schizonte is slightly shorter than that of the control group animals (P<0.05). In addition, the maturity of the therapeutic group histotype schizonte is obviously lower than that of the control group (P<0.01).

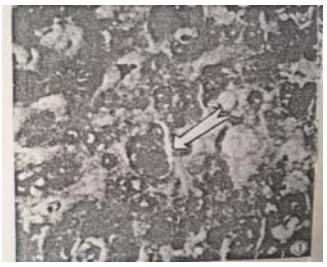

Fig 1: The shape of the therapeutic control group's histotype schizonte×1000

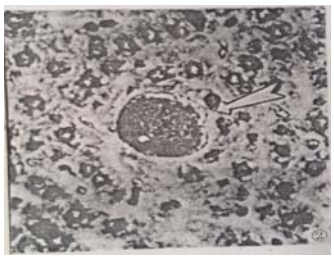

Fig 2: The shape of the haematoxylin-Eosin stain × 1000

Table 2: The effect of *Chrysanthemum moroifolium Ramat* ethylacetate extract on P. falciparum

group	extract concentration in cultivation medium (μg/ml)	Parasitemia (%)				
		1.	2.	3.	4.	5. day
control group	0	1.2	3.0	6.1	8.0	10.9
therapeutic	50	1.1	2.0	3.0	3.9	7.2
group	70	1.2	2.2	2.9	3.3	5.5
	80	1.1	1.9	2.2	1.5	1.8
	90	1.2	1.6	2.0	1.7	1.9
	100			2.2	1.8	1.0

In the cell cultivation, the *Chrysanthemum morifolium Ramat* ethylacetate extract also exhibits antimalaria action. With an increase in the concentration of this extract in the cultivation medium, the infectiosity of the erythrocytic plasmodia in

medium will decrease as a result. When the consentration of the extract in the medium is raised to 100μg/ml, the parasitemia of the therapeutic group will have dropped to 1.0% on the fifth day while that of the control group has in increased to 10.9%, showing that the *Chrysanthemum morifolium Ramat* ethylacetate extract passesses good anti-P. Falciparum action.

4. Discussion

It has been found during experiments on animals that the *Pasta ulmi* extract has the antmalarial action on P. vinckei, and the action of its lipophil extract is even stronger. As no record of *Pasta ulmi's* antimalaria has ever been seen, it is believed to be a new discovery. Therefore, further observation of the therapeutic effect of the extract on P. berghei [5] of the chloroquine sensible strain and chloroquine resistant strain is made. It is found that the extract is effective for both. In recent years, it is mainly P. falciparum that has been found to generate resistance to medicine, which has resulted in large casualties of patients. Does the *Pasta ulmi's* lipophil extract have any effect on P. falciparum? It has been proved by subsequent experimental results that, when the concentration of *Pasta ulmi* lipophil extract C3 in the cultivation medium is raised to 10μg/ml cultivation medium, on the fifth day in the course of cultivation, all erythrocytic plasmodia in the cultivation medium are cleared. Prepared by processing *Pasta ulmi* and plants of the chrysanthemum family, *Pasta ulmi* became known for its antimalaria action which made *Pasta ulmi* and plants of the same genus the objects for antimalaria tests but to no avail. Later, observation of the antimalaria effect of several plants in the chrysanthemum family has been performed. Of the plants mentioned, one exhibits the same antimalaria effect as *Pasta ulmi.* According to the certification by Hubei Provincial Academy of Traditional Chinese Medicine, the said chrysanthemum family plant is traditional Chinese medicine *Chrysanthemum morifolium Ramat.* Therefore, it can be considered that the antimalaria action of *Pasta ulmi* originates in *Chrysanthemum morifolium Ramat.* The object of investigation from now on will be shifted from *Pasta ulmi* to *Chrysanthemum morifolium Ramat.* In the observation of the effect of *Chrysanthemum morifolium Ramat* chloroform extract on histotype schizontes, after young white rats are given an injection of parasite sporozoites, the sporozoites that invaded the liver cells should rightfully develop into histotype schizontes. But under the action of the *Chrysanthemum morifolium Ramat* extract, both the maturity and diameter of the histotype schizontes are smaller than those of the control group, showing that the *Chrysanthemum morifplium Ramat* chloroform extract has a suppressing action on the histotype schizontes that is helpful to preventing relapse into malaria. It is noticed that pernicious malaria has in recent years gradually developed a medicine resisting ability to traditional antimalaria drugs. Therefore, it is an urgent task for malaria research people to seek new antimalaria drugs. In the circumstances, the discovery of the antimalaria action of *Chrysanthemum morifolium Ramat* appears to be of even greater value. Following on the heels of Artemisia annua, *Chrysanthemum morifolium Ramat* is another traditional Chinese medicine with antimalaria effect ever discovered in China's researches on traditional antimalaria medicines.The difficulty of research on traditional Chinese medicine lies in the very frequently seen cases where one and the same name is given to different drugs or different drugs may share one and the same name. So it is of utmost importance to first have one or more traditional Chinese medicines certificated by authoritative traditional Chinese medicine organs and repeatedly experimented and observed prior to their use for research and to declaring them effective or ineffective.

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