

Investigations of *Artemisia Annua* and *Artemisia Sieberi* Water Extracts Inhibitory Effects on β -Hematin Formation

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Introduction

Malaria is the most prevalent infectious disease in the world, killing 1-2 million people each year. New drugs are urgently needed to treat drug-resistant strains of malaria. In a previous study we found that extracts from *Salvia palestina* leaves inhibited the formation of β -hematin with efficiency similar to that of chloroquine.

Aim

The objective of this study was to investigate the effect of two plant extracts on hemozoin formation. A comparison between the efficiency of aqueous extracts or infusions of *Artemisia annua* from Luxembourg and *Artemisia sieberi* from Palestine in inhibiting β -hematin formation was done.

Materials and Methods. Water infusions of *Artemisia annua* and *Artemisia sieberi* from Luxembourg and Palestine, were used in this study. A semi-quantitative *in vitro* method, based on the inhibition of ferriprotoporphyrin IX (FP) bio-mineralisation developed by Deharo *et al.* [Deharo E *et al. Exper. Parasitol.* 2002, **100**:252-256.], was used to reveal the differences in antimalarial activity of both plants.

Extraction of plant components

A. Preparation of *Artemisia annua* extract (Method A)

Dried leaves and stems were separately ground into coarse powder; extraction was performed by soaking (1:10) wt./vol. of dried plant part, in distilled hot water at 90°C, and then left for 20 to 24 hours at room temperature. The extract was filtered using MN 615.0110 mm filter paper. The crude water extract was obtained after the solvent was evaporated at 60-80°C under reduced pressure using (IKA WEREK RV06-ML) rotary evaporator, followed by lyophilization using (Labconco freeze drier) until constant weight was achieved. The final dried extract was stored in opaque bottles and kept in a desiccator until use. The results of Figures 1 and 4 correspond to this extraction method.

B. Infusion of *Artemisia annua* (Method B)

2 g of the plant material were soaked in 150 ml of distilled hot water at 90°C, left for 20 minutes at room temperature, then filtered using MN 615.0110 mm filter paper. All other figures correspond to this extraction method

Results: Although it was found that the *Artemisia sieberi* leaf tea infusion was less effective than that of the *Artemisia annua*, the stem infusion of *Artemisia sieberi* was found to be better than that of *Artemisia annua* stems. Results obtained with infusions prepared with tap or well water may be different from results obtained in the laboratory with distilled water. *Artemisia annua* leaf infusions prepared using salt water (0.5g salt/150ml water) had higher efficiency in inhibiting β -hematin formation than those infusions done with distilled water. Mixing equal amounts of *Artemisia annua* leaf and *Artemisia sieberi* stem water extract showed an increase in their inhibitory effect on β -hematin formation. An important finding in this investigation was that the *Artemisia annua* lyophilized extracts lost activity with time, which may have an impact not only on *in vitro* laboratory results but also on *in vivo* treatment efficiency obtained with old extracts.

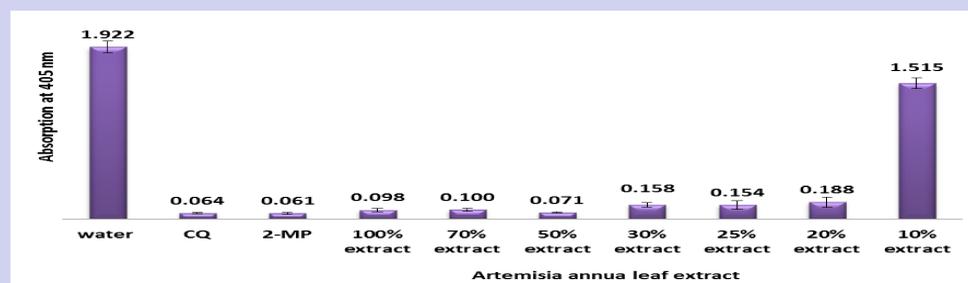


Figure 1: Column diagram representing the efficacy of potential antimalarial drug *Artemisia annua* leaf water extract using method B, compared to the negative and positive controls: CQ-chloroquine 0.1mg/ml and 2-MP (2-mercaptopyrimidine)1mg/ml, showing the absorption values of dissolved β -hematin (alkaline hematin) at 405 nm using ELISA reader, according to E. Deharo semi-quantitative method. The absorption is inversely proportional to drugs efficiency, the lower the absorption is, the drug is considered to be more efficient. Each result represents the average of 16 individual experiments.

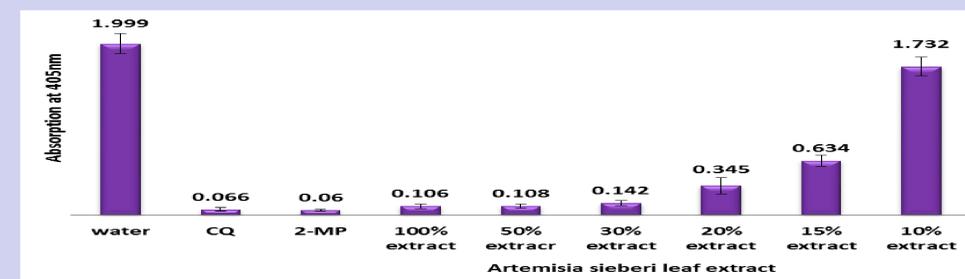


Figure 2: Column diagram representing the efficacy of potential antimalarial drug *Artemisia sieberi* leaf water extract using method B, compared to the negative and positive controls: CQ-chloroquine 0.1 mg/ml and 2MP- 2-mercaptopyrimidine 1mg/ml, showing the absorption values of dissolved β -hematin (alkaline hematin) at 405 nm using ELISA reader, according to E. Deharo semi-quantitative method. The absorption is inversely proportional to drugs efficiency, the lower the absorption is, the drug is considered to be more efficient. Each result represents the average of 16 individual experiments.

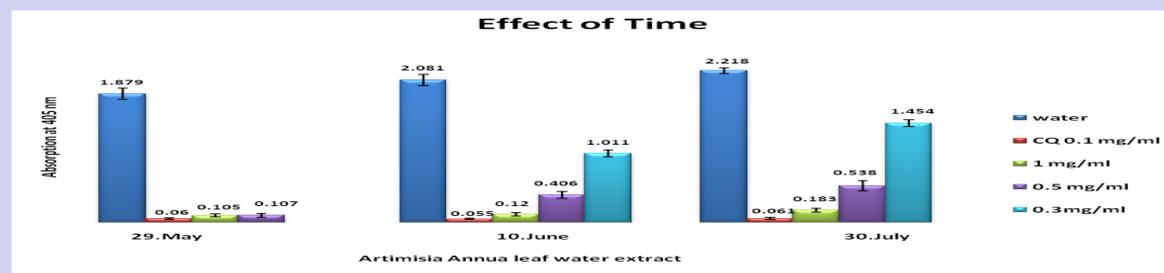


Figure 3: Column diagram representing the effect of time on the efficacy of *Artemisia annua* leaf water extract using method A, to inhibit β -hematin formation, compared to the negative (water) and positive (chloroquine) controls: CQ- chloroquine 0.1 mg/ml, showing the absorption values of dissolved β -hematin (alkaline hematin) at 405 nm using ELISA reader, to E. Deharo semi-quantitative method. Each result represents the average of 16 individual experiments.

Conclusion: In light of this finding it might be advisable to use *Artemisia annua* in the form of dried leaf powder and not in the form of extracts or infusion. Stored in dry, ventilated conditions the plant keeps its properties for many years.

Acknowledgment

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