# Hemolytic activity of different herbal extracts used in Algeria

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**ABSTRACT**

**Purpose**: The membrane of erythrocyte can be affected by consumption of bioactive compounds from herbs and medicinal plants. Hemolytic activities of nine extracts from different species on human erythrocytes were investigated. **Methods**: The toxicity of methanolic extracts of nine plants from arid and sub-arid area on human erythrocytes was measured by in vitro hemolytic assay. The absorbance of hemoglobin release was read by spectrophotometer at 540 nm. **Results**: our results show that hemolytic activity in all extracts is not more than15% and not significant alteration on erythrocyte membrane was observed at low concentrations while hemolytic activity is concentration-dependent. Hemolytic effect was amplified at increased concentration and it is function of extract. The higher hemolytic activity is showing with extract from the Saharan species *Morettia canescens* (14.80%). In addition, none hemolytic activity was observed against human erythrocytes with *Paronychia chlorothyrsa* extract. The present study show that majority of extracts of different plants manifest hemolytic activity < 15%. **Conclusion**: These results explain that parameters of red blood membrane cell are not altered if therapeutic formulations from these plants are used at low concentration, but a great attention will take with greatest concentrations.

**Keywords:** medicinal plants, bioactive compounds, toxicity, erythrocyte, hemolytic activity

## 1. INTRODUCTION

The practice of traditional medicine using medicinal plants is as old as the origin of man [1]. Substances found in medicinal plants are known as the active principles. These compounds have been extracted and used in different forms such as infusions, syrups, decoctions, infused oils, essential oils and creams [2]. Plant-derived natural products such as flavonoids, terpenes, alkaloids, anthraquinones, saponins, tannins, steroids, lactones and volatile oils received considerable attention in recent years due to their diverse pharmacological properties, including cytotoxic and chemo-preventive effects [3]. Active compounds of plants are used in folk, traditional and alternative medicine to treat diseases like cancer, cardiovascular, Alzheimer's, Parkinson's disease [4-7] and have antioxidant; anti-inflammatory, antidiarrheal, antimicrobial, Anti-parasitic, Antiviral activities, etc. [8-13]. Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risks are evaluated [1].

Many plants contain chemical substances that might have a hemolytic or anti-hemolytic effect on human erythrocytes. Several reports indicate that the membranes of human erythrocytes from blood types have varying stability as determined from the mean corpuscular fragility [14]. Plant extracts can positively affect the red cell membrane [15] and many plants have serious adverse effects, which include induction of hemolytic anemia. Therefore, many of the commonly used plants need to be evaluated for their potential hemolytic activity.

The present study was carried out the hemolytic activity of various extracts from different species of northwest and south of Algeria; the selected plants were: *Calotropis procera* (Ait.), *Paronychia argentea* (Lam.), *Paronychia chlorothyrsa* (Murb.) *Morettia canescens* (Boiss.), Haloxylon *scoparium* (Pomel), *Thymelaea hirsuta* (L.), *Tamarix aphylla* (L.) Karst., *Daphne gnidium* (L.) and *Arthrophytum schmittianum* (Pomel).

## 2. MATERIAL AND METHODS

### *2.1. Plant material*

All plant material was collected from different area. *Morettia canescens* (Aerial part), *Tamarix aphylla* (leaves), *Calotropis procera* (leaves) were collected from Algerian Sahara (*Adrar*), *Paronychia chlorothyrsa* (aerial part) from *Tiaret*, *Paronychia argentea* (aerial part) from *Mecheria*, *Thymelaea hirsuta* (leaves) from *Mascara*, *Haloxylon scoparium* (aerial part) from *Ghardaia* and *Arthrophytum schmittianum* (aerial part) from *Tebessa* while *Daphne gnidium* (leaves) collected from North West of Algeria (*Tlemcen*).

### *2.2. Preparation of plant extracts*

For each plant, dried and powdered material was extracted with water-methanol (30:70, V/V) for 48h. The extracts were filtered and dried using Christ-Alpha 1-4 Lyophilizator. The lyophilized dry powder was weighed and kept in a dry place until being used for the hemolytic test.

### *2.3. Hemolytic Activity*

Methanolic extracts of nine species was assayed on human erythrocytes (O blood groups) following the method of Malagoli [16]. The human blood samples were obtained from healthy volunteers. The blood was centrifuged at 5,000 rpm for five minutes. 2 % erythrocyte suspension was prepared in sterile phosphate buffer saline for hemolytic study.

The hemolytic activity of the crude extract was tested under in vitro conditions, for each plant sample, various concentrations (50–500 µg/ml) of extracts were added to 0.85% NaCl solution and then received a 2% suspension of human erythrocytes. After 30-min incubation at room temperature, cells were centrifuged and the supernatant was used to measure the absorbance of the liberated hemoglobin at 540 nm. Two controls were prepared without extracts; negative control received sterile phosphate buffer saline, while positive control received 0.1% Triton X-100.The average value was calculated from triplicate assays.

Hemolysis percentage for each sample was calculated by dividing sample's absorbance on positive control absorbance (complete hemolysis) multiplied by 100 [17].

### *2.4. Statistical analysis*

Experimental data were analyzed with three replicates for analysis of variance. Statistical analysis was done by One Way ANOVA. The p values less than 0.05 were considered to be statistically significant.

## 3. RESULTS

The yields of extraction are: 13.09% (*A. schinithranum*), 6.05% (*C. procera*), 9.65% (*D. gnidium*), 17.29% (*H. scoparium*), 28.70% (*M. canescens*), 7.88% (*P. argentea*), 14.97% (*P. chlorothyrsa*), 6.75% (*T. aphylla*), and 4.88% (*T. hirsuta*).

Hemolytic assays were performed because compounds possessing potent biological activity may not be useful in pharmacological preparations if they possess hemolytic effect. In addition, these data also may reveal some information about the mechanism of cytotoxicity.

In vitro hemolytic activity on human erythrocytes of various concentrations extracts obtained from aerial part of *Paronychia argentea*, *Paronychia chlorothyrsa*, *Morettia canescens*, *Haloxylon scoparium*, *Arthrophytum schmittianum* and leaves of *Calotropis procera*, *Thymelaea hirsuta*, *Tamarix aphylla*, *Daphne gnidium* was performed. The total hemolysis was obtained using Triton X-100 (0.1%) and 0% hemolysis was obtained with buffer. Hemolytic activity of various crude extracts is shown in Figure 1-4. Each concentration shows the mean of hemolysis percentage repeated in three experiments. The hemolysis induced by extracts in red blood cells was concentration-dependent but all extract showed lower hemolytic effect on human red blood cell on all concentrations tested exception increased hemolytic level with extract from *Morettia canescens.*

None of the concentration test of *Paronychia chlorothyrsa* extract possesses any hemolytic activity against human erythrocytes. The effect of methanolic crude extract of this species on blood erythrocyte membrane showed not changes in hemolytic activity when concentration varied from 50µg to 500µg per milliliter. The hemolytic percentage 0.056%, 0.098%, 0.231 and 0.452% were obtained for a dose of 50µg/ml, 100µg/ml, 250µg/ml and 500µg/ml respectively. These values were statistically considered not significant when we compared with zero value ($p > 0.05$). In addition, *Paronychia argentea* extract present significant hemolytic effect ($p < 0.05$) at all concentration but this effect is very low not exceeding 3%.

The species of genus *Paronychia*; *P. argentea* and *P. chlorothyrsa* are called by Algerian's population « *ferschn'dah* » and in Arabic are known under the name of "Arabic tea". These species are good antiseptic for respiratory and urinary systems. If compared hemolytic profile between the two species shown in figure 1; 2; 3 and figure 4, it is preferable for human health to use *Paronychia chlorothyrsa* in the traditional medicinal preparations.

*Arthrophytum schmittianiun* extract has no significant changes in percentage hemolytic values determined at the dose levels from 50 to 250µg/ml. the compared means with control are not significant ($p > 0.05$) but, a slight increase in hemolysis (1.42%) was observed at high dose 500µg/ml. At this level, *Arthrophytum schmittianum* revealed significant toxicity. Furthermore, not toxicity on erythrocytes was observed with *Haloxylon scoparium* extracts at the concentration $\leq$ 100µg/ml. Also, the extracts of *Thymelaea hirsuta*, *Calotropis procera* and *Morettia canescens* have not hemolytic effects ($p > 0.05$) at 50µg/ml.

*Tamarix aphylla* and *Daphne gnidium* given 6.568 % and 7.060% hemolysis at 500µg/ml. *Daphne gnidium* and *Tamarix aphylla* provoked toxicity but less than *Morettia canescens* while a great hemolytic effect on erythrocyte cell membrane was caused by this species (14.80% hemolysis).

## 4. DISCUSSION

The erythrocyte model has been widely used as it presents a direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity. Another advantage of erythrocytes model is that blood is readily available and that cells are easy to isolate from the blood; moreover, its membrane has similarities with other cell membrane [18]. Erythrocytes have been used as a model system by a number of workers for the study of interaction of drugs with membranes [19-22]. Hemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer. This hemolysis relates to concentration and potency of extract. Furthermore the hemolytic activity of each extract is related to their chemical composition. *Paronychia chlorothyra* has not effect and not affect stability of the erythrocyte membrane; these data suggested the non–toxic effect of the extract thus making it suitable for the preparation of drugs involved in the treatment of various diseases. Also, *Arthrophytum schmittianum* is not given toxicity on human erythrocytes at low concentration less than 500µg/ml. This species; called "Remt" is used by Algerian's population for treat cancer.

The remarkably observation is for *Calotropis procera*, medicinal species with toxic effects showed low hemolytic activity on human erythrocyte compared to others considered not toxic. It possible that the cytotoxic activity was not only or not related to lytic properties or membrane instability induced by the extracts [23], but it do to another mechanism. This species produce cardiac principles [24] and present a great medicinal interest, it was used for treat intestinal worms, cutaneous diseases, asthma, bronchitis, anorexia, inflammations and tumors. Also, a similar observation was shown with *Thymeleae hirsuta*, species considered as toxic for animals, the extract of this species has showed low hemolytic effect (4.735%) at high concentration. This species is commonly known as "Methnane" has been used in traditional medicine for its antiseptic, antimelanogenesis, antihypertension and hypoglycaemic properties [25-27]. Furthermore, The Algerian Bunge; *Haloxylon scoparium* contain a great number of alkaloids like cargenine and *N*-methylisosalsoline [28] which display potent antimicrobial, antimalarial, cytotoxic and anti-HIV activities [29].

Our results showed more ability of human erythrocyte to hydrolysis in presence of extracts from *Daphne gnidium*, *Tamarix aphylla* and *Morettia canescens*. It has reported that the methanol extract from the leaves of *Daphne gnidium* possess antileukemic, antibacterial, antimycotic activities [30] and *Tamarix aphylla* are used in folk medicine to evacuate coagulated blood and trait hematomas.

## 5. CONCLUSION

In general, the results of the present study show that majority of extracts of different plants manifest low hemolytic activity but a great attention will take with greatest concentrations.

It is necessary to be vigilant for the potential secondary effects generated by plant therapies because traditional experiment is not very useful when it is question of evaluating the risk. Moreover, administration of the plant by *per os* or others ways requires some precautions and the determination of hemolytic effect of the extracts becomes necessary but not taken only. It is very important to associate all the others in vitro and especially in vivo methods on animal models also in the case of a prolonged administration of the plant and or use strong amounts, it would be advised to proceed to the follow-up of the hematologic parameters, which will make it possible to prevent the risks of a possible anemia.

## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

## ACKNOWLEDGEMENTS

This work was financially supported by Laboratory of Natural product, ministry of higher education and scientific research, Algeria

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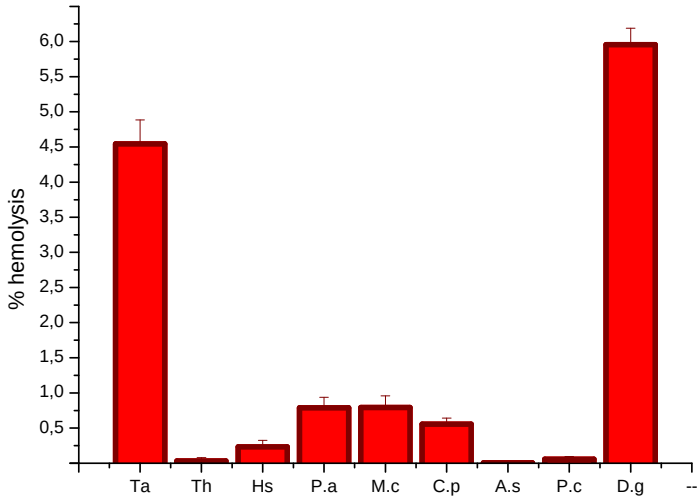


Figure 1: hemolytic effect of the nine extracts at the concentration of 50µg/ml (Ta: Tamarix aphylla, Th: Thymelaea hirsuta, Hs: Haloxylon scoparium, Pa: Paronychia argentea, Mc: Morettia canescens, Cp: Calotropis procera, As: Arthrophytum schmittianum, Pc: Paronychia chlorothyrsa, Dg: Daphne gnidium)

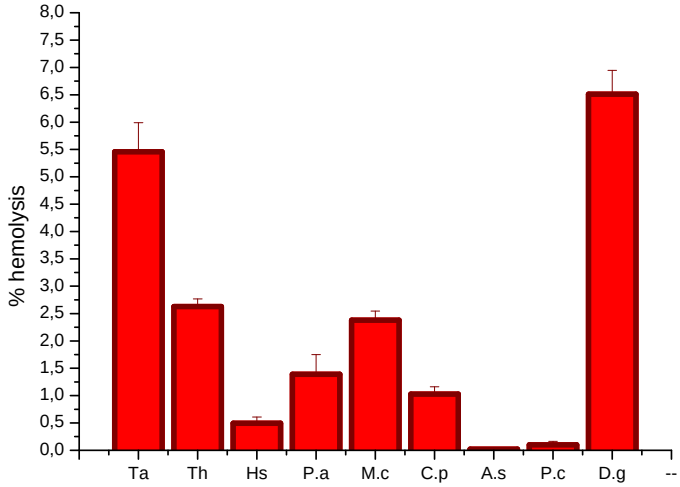


Figure 2: hemolytic effect of the nine extracts at the concentration of 100µg/ml (Ta: Tamarix aphylla, Th: Thymelaea hirsuta, Hs: Haloxylon scoparium, Pa: Paronychia argentea, Mc: Morettia canescens, Cp: Calotropis procera, As: Arthrophytum schmittianum, Pc: Paronychia chlorothyrsa, Dg: Daphne gnidium)

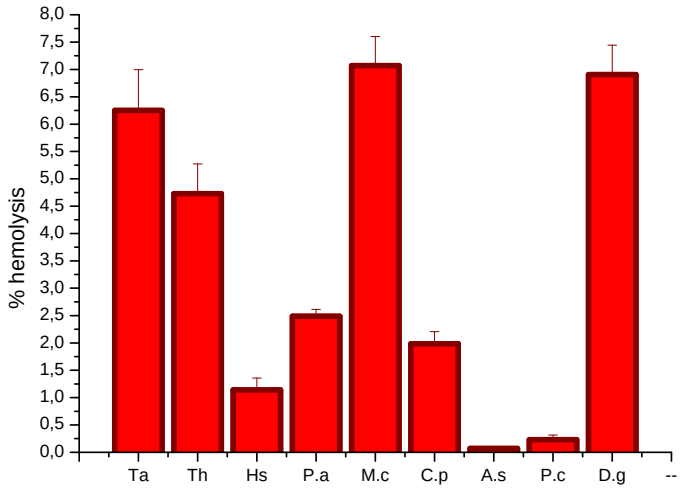


Figure 3: hemolytic effect of the nine extracts at the concentration of 250µg/ml (Ta: Tamarix aphylla, Th: Thymelaea hirsuta, Hs: Haloxylon scoparium, Pa: Paronychia argentea, Mc: Morettia canescens, Cp: Calotropis procera, As: Arthrophytum schmittianum, Pc: Paronychia chlorothyrsa, Dg: Daphne gnidium)

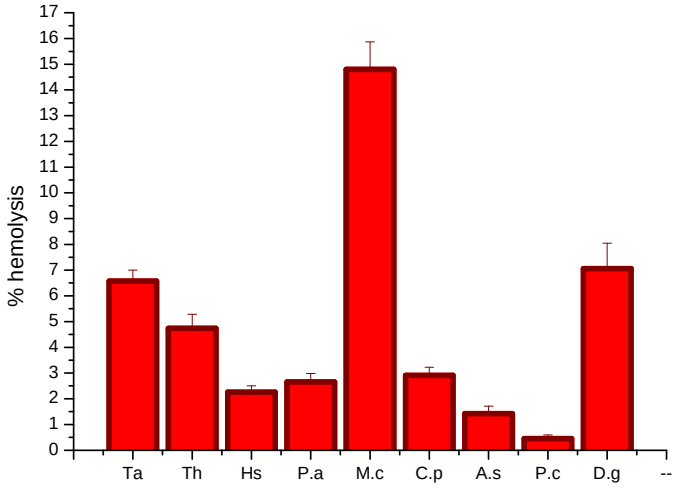


Figure 4: hemolytic effect of the nine extracts at the concentration of 500µg/ml (Ta: Tamarix aphylla, Th: Thymelaea hirsuta, Hs: Haloxylon scoparium, Pa: Paronychia argentea, Mc: Morettia canescens, Cp: Calotropis procera, As: Arthrophytum schmittianum, Pc: Paronychia chlorothyrsa, Dg: Daphne gnidium)