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Evaluation of Biological Activities of *Chamaeleo chamaeleon*: A Reptile Used in Traditional Folk Medicine in Algeria

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Abstract

In the El-Oued region of the Algerian desert, tonsillitis, the most serious of infections, is cured with the common chameleon, *Chamaeleo chamaeleon*. Our study's main objective was to demonstrate its importance through aspirational science, in which we evaluated the aqueous extract of dry *C.Chamaeleon* powder's hemolysis, anti-inflammatory, and antibacterial properties. After preparing dry, *C.Chamaeleon* powder was examined using a scanning electron microscope to detect metals. The aqueous extract was then analyzed with an infrared analyzer to determine the functional groups following extraction. We also examined the extract's capacity to induce hemolysis, its antibacterial activity against six bacterial strains, and its anti-inflammatory activity using the egg albumin denaturation method. The findings of the hemolysis assay showed low percentages at high doses until the ratio reaches 20.7% at 1 mg/ml, compared to sodium dodecyl sulfate, which showed very high rates of hemolysis in the presence of low concentrations up to 90% at 0.25 mg/ml, indicating that the extract is non-toxic to red blood cells. The anti-inflammatory activity test yielded good results and revealed ratios of 12.82%, 22.07%, and 38.2% for the extract at doses of 0.4, 0.6, and 0.8 mg/ml, respectively. And we also found that the extract was anti- all the bacterial strains studied at a dose of 100 mg/ml. According to the results of our study, *C.Chamaeleon* aqueous extract has significant medical value in treating infectious and inflammatory illnesses since it contains several minerals, peptides, and vitamins.

Keywords: Zootherapy, Chamaeleo chamaeleon, Hemolysis, Anti-inflammatory, Antibacterial

Introduction

Since ancient times, man has been searching in nature for sources of survival, such as plants, animals, marine organisms, and microorganisms, which he used for his food and drink, and even in treating many diseases that afflict him. According to fossil records, humans' use of plants as medicine goes back at least 60,000 years (Yuan *et al*., 2016). Traditional medicine, as described by the World Health Organization, is defined as various knowledge and applications based on the original experiences of different cultures, which are used to maintain physical or mental health by preventing or treating diseases (Abdullahi, 2011). Humans use alternative medicine plants, animals, and minerals to produce remedies, and they have been firmly integrated into contemporary societies, especially phytotherapy and zootherapy (Santos *et al*., 2019). Zootherapy is the use of animals or their products in the treatment of many diseases that affect humans. It was used in ancient societies and is still applied today (Alves & Rosa, 2005).

Reptiles are among the most commonly employed animal species in traditional folk medicine. Their significance in healing and/or preventing illness has been documented in various social-cultural contexts worldwide (Alves *et al*., 2012). Chameleons are highly specialized and mostly arboreal lizards characterized by a suite of derived characters. They are also important ingredients in the preparation of curative, protective, and preventative medicines for disease immunity, protection against bad luck and witches, aphrodisiacs and potency, and to bring good healing (Williams & Whiting, 2016).

After slaughtering and salting, the dried chameleon is known to be used to treat several ailments endemic in the El-Oued region of the Algerian desert. Even doctors in the region urge their patients to take it for the emergence of its guaranteed effectiveness, where it has been employed in various ways, most notably in the treatment of tonsillitis. It was also used in the treatment of cough and thyroid diseases.

Chamaeleo chamaeleon is the most common type of chameleon, also known as the Mediterranean chameleon. However, it is an oviparous reptile that lives in hot regions and feeds largely on insects (Keren-Rotem *et al*., 2006). It has many names according to the region, as it is called in the Oued Souf region of El-Bouwayh.

Unlike plant extracts, animal extracts lack scientific evidence to demonstrate their biological usefulness. Hence our research aimed to study the biological activity of the aqueous extract of dry *Chamaeleo chamaeleon*. The hemolysis assay and anti-inflammatory, the antibacterial activity of the aqueous extract of dried C.Chamaeleon were all tested in this work.

Materials and Methods

Aqueous Extraction and Chemical Analysis

Samples of *C. Chamaeleon* were purchased from the herbalist, where they were already ready-dried following the traditional

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manner used by the people of the El Oued region. Then it is ground and stored for analysis. The material was extracted for 6 hours with distilled water Using the Soxhlet device. The C.Chamaeleon aqueous extract (AQ) was concentrated in a rotary evaporator at 45 °C before being dried in an oven and weighed to determine the extraction yield. The extracts were stored at –10°C until analysis.

The *C.Chamaeleon* powder and ash samples were examined using an Energy-dispersive X-ray analysis along with Scanning Electron Microscope (SEM-EDX) to detect metals, and the AQ was reviewed under an infrared analyzer to determine their functional groups

Hemolysis Assay

According to Vinjamuri *et al*. (2015), the hemolysis test was performed in the following steps: We collected 5 ml of blood from healthy volunteers in EDTA tubes to prevent coagulation and centrifuged at 1000 rpm for ten minutes, then removed the plasma and the white buffy layer completely, and after that wash erythrocyte three more times with 1X PBS (phosphate-buffered saline, pH 7.4) for five minutes. The washed blood cells are stored at 4°C and used for a period not exceeding 6 hours. 50 µl of 10 dilutions (100 µl of erythrocyte suspension: 900 µl of 1X PBS) of the erythrocyte suspension were mixed with 100 µl of the extract at different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg/ml), 100 µl of 1X PBS was used as a negative control, and 100µl of SDS as a positive control, and incubated the mixture was placed in a 37°C water bath for 60 minutes. The mixture volume delivers to 1 mL by adding 850 µL of 1X PBS. Finally, it was centrifuged at 300 rpm, later the absorbance of hemoglobin in the supernatant was measured at 540 nm, and the percentage of hemolysis was calculated according to the following equation:

$$\% \text{ Hemolysis inhibition} = 100-(\text{Sample}/ (\text{Control})) \times 100 \quad (1)$$

Anti-Inflammatory Activity

According to Chandra *et al*. (2012), we chose the egg albumen denaturation method with some modifications to test the anti-inflammatory. We mixed 0.4 ml of egg albumin (from fresh hen's eggs) with 0.8 ml of PBS (phosphate-buffered saline, pH6.4) and 2 ml of chameleon extract at different concentrations (0.4, 0.6, and 0.8 mg/ml), and the same volume of distilled water as a control. Then the mixture was incubated at 37 °C for 15 minutes and immediately placed in a water bath at 70 °C for five minutes. After cooling, it was centrifuged at 3000 rpm for 10 minutes. The absorbance was measured at 660 nm. The acid acetylsalicylic was used as a reference drug, and to calculate the percentage of protein denaturation inhibition, we use the following formula:

$$\%\text{Inhibition} = ((\text{Ac-As})/\text{Ac}) \times 100 \quad (2)$$

Antibacterial Activity

The antibacterial activity of the aqueous chameleon extract was determined using the diffusion on agar medium or aromatogram method. The aromatogram is a method based on the antibiogram that allows researchers to measure an extract's inhibitory action by measuring the diameter of inhibition around a disc impregnated with it (Anthoney Swamy *et al*., 2014). Four concentrations (100, 75, 50, 25 mg/ml) of extract diluted in DMSO (dimethyl sulfoxide) were tested against six referenced strains (Bacillus subtilis, Listeria innocua, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhimurium)

A disc of 6 mm in diameter made of sterile Whatman paper is impregnated with the extract and then placed in the middle of a Petri dish 90 mm in diameter containing a 4 mm thick "Mueller Hinton" agar medium (Selam *et al*., 2022), previously inoculated by swab with the inoculum. The latter is prepared at a concentration of 0.5 Marc Ferland, from 106 to 108 CFU mL-1 for each strain. Discs impregnated with DMSO and antibiotics (Azithromycin, Gentamicin) are applied to the control dish, and three repetitions are carried out for each test.

The Petri dishes are incubated for 24 hours at 37°C, and the reading is carried out by measuring the inhibition zone diameter, resulting in a translucent halo around each disc. The presence or absence of a halo would explain the germs' sensitivity or resistance to the extracts tested; according to a symbolic notation scale ranging from – to +++ and whose reading, according to Boughendjioua (2017), is done as follows:
Ø < 10 mm: aqueous extract (AQ) without inhibitory action (-).
16 > Ø ≥ 10 mm: AQ with an intermediate (+) inhibitory action.
25 > Ø ≥16 mm: AQ with significant inhibitory action (++).
Ø≥25 mm: AQ with a very effective inhibitory action (+++).

Statistical Analysis

The data were analyzed using SPSS-26 and are presented as mean ± SEM. EXCEL (version 2010), used to calculate the IC50 values.

Results and Discussion

Extraction Yield

The following formula was used to compute the extraction yield (%): Extraction yield (%) = (W1/W2) ×100. W1 is the weight of the extract after evaporating the solvent, and W2 is the sample's dry weight, where the average yield result for three extractions is 14.36±1.61%.

SEM-EDX Device and Infrared Analyzer Results

The results of different mineral contents in the Chamaeleo chamaeleon powder and ash using the Energy-dispersive X-ray analysis and a Scanning Electron Microscope (SEM-EDX) are shown in **Table 1**. The results demonstrate that C.Chamaeleon powder contains a high concentration of carbon, a medium concentration of oxygen and nitrogen, and a trace amount of sulfate. *C.Chamaeleon* ash contains rhodium, calcium, phosphate, potassium, and zirconium.

Table 1. Some minerals in the chameleon powder and ash

Element	Atomic Concentration	Weight Concentration
Powder of *C.Chamaeleon*		
Carbon (C)	60.32	51.79
Oxygen (O)	15.54	17.78

Sulfate (S)	0.24	0.56
Nitrogen (N)	18.87	18.90
Ash of *C.Chamaeleon*		
Rhodium (Rh)	2.71	10.24
Calcium (Ca)	5.06	7.44
Phosphate(P)	4.02	4.57
Potassium(K)	1.67	2.39
Zirconium (Zr)	0.44	1.46

The absorption spectra exhibited seven peaks of absorbance **(Figure 1)**. The broad peak at 3335 cm-1 represented the presence of alcohol (O–H stretch) (Başaran *et al*., 2022), and secondary amines (N–H stretch). In addition, the peak at 2960 cm-1showed the presence of alkane and carboxylic acid (O-H stretching). The peak at 2150 cm-1 represented the presence of alkyne (CΞC). The strong peak at 1610 cm-1 exhibited the presence of aromatic (C=C stretch). The frequency range of 1400-1000 cm-1 represented 3 peaks and showed the presence of nitro (N-O stretching), alkane (C-H stretching), ester (C-O stretching), and amines (C-N stretching).

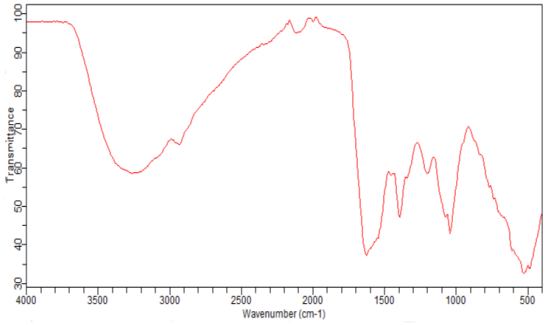

Figure 1. Infrared spectrums of *C.Chamaeleon* aqueous extract functional group

The extract was run through FT-IR to separate the functional groups of the components based on the peak ratio. The analysis revealed the existence of the following functional groups: O–H, N–H, CΞC, C=C, N-O, C-H, C-O. According to Stuart (2004), the presence of this functional group predicts that the extract can contain various compounds like proteins, vitamins, and nucleic acids.

Hemolysis Assay

In the presence of large quantities of the extract (0.2-1 mg/ml), the percentage of hemolysis of the aqueous extract is relatively poor until the ratio reaches 20.7% at 1 mg/ml, as shown in **Table 2**. Compared to sodium dodecyl sulfate (SDS), which showed very high hemolysis rates in the presence of low concentrations (0.05 - 0.25 mg/ml) and up to 90% at 0.25 mg/ml. However, this percentage increases with increasing concentrations until it reaches 20.7 at 1 mg/ml, proving that the aqueous extract of dry chameleon is not toxic to red blood cells at medium or weak concentrations (Fan *et al*., 2022).

Table 2. Percentages of hemolysis of the aqueous extract (AQ) of *C.Chamaeleon* and sodium dodecyl sulfate (SDS)

C mg/ml	% Hemolysis of AQ	C mg/ml	% Hemolysis of SDS
0,2	5,5	0,05	74,51
0,4	9,26	0,1	78
0,6	13,2	0,15	81,87
0,8	16,4	0,2	85,55
1	20,7	0,25	90

Anti-Inflammatory Activity

The results of anti-inflammatory ratios for the aqueous extract of a dry chameleon and also Acid Acetilsalicilic at concentrations of 0.4, 0.6, and 0.8 mg/ml are shown that the anti-inflammatory activity increases with increasing concentrations, the results gave respectively, ratios of 12.82%, 22.07% and 38.2% for the extract. And the ratios of 38.36%, 66.81%, and 89.46% for Acid Acetilsalicilic. As a result, the extract exhibits a strong anti-inflammatory action due to its ability to prevent the denaturation of egg albumin. The anti-inflammatory activity of the extract is because it may contain vitamin B groups, especially vitamin B6, which has anti-inflammatory properties, and vitamin B12 (Hosseinzadeh *et al*., 2012; Moshiri *et al*., 2018), which provides anti-denaturation protection for egg albumin.

Antibacterial Activity

Compared to antibiotics (Azithromycin, Gentamicin), the aqueous extract of dried C.Chamaeleon is efficient against all tested bacterial strains at a concentration of 100% and effective against L. innocua, B. subtilis, P. aeruginosa, and E.coli at a concentration of 75% **Table 3**. But at a concentration of 50%, it is effective against P. aeruginosa and E. coli. It was solely effective against P. aeruginous at a concentration of 25%.

The antibacterial results showed that the extract has efficacy against bacteria. This antibacterial activity may be due to the presence of calcium ions (Ca2+), which disrupt the cytoplasmic enzymes of microorganisms, causing toxic effects on bacterial cells. The presence of peptides that interact with bacteria causes their autolysis (de Magalhães Silveira *et al*., 2011; Park *et al*., 2022). Vitamin B2 contributes to its antibacterial activity, especially for Gram+. According to Teyssier *et al*. (2015), one of the basic compounds that are found in the chameleon skin which interfere with color transformation is nanocrystals, including guanine nanocrystals, which have special important biological activities, where nanocrystals that bind to the amino groups on the surface of bacteria and cause them to be killed (Fang *et al*., 2018; Peng *et al*., 2018; Alawad *et al*., 2021).

Table 3. Antibacterial activity of *C.Chamaeleon* aqueous extract (AQ) compared to antibiotics (Azithromycin, Gentamicin) and DMSO

	Gram-positive		
	B. subtilis ATCC 6633	*L. innocua* CLIP 74915	*S. aureus* ATCC 6538
AQ 100%	+	++	+
AQ 75%	+	+	-

AQ 50%	-	-	-
AQ 25%	-	-	-
Azithromycin	+++	++	++
DMSO	-	-	-
	Gram-negative		
	P. aeruginosa ATCC 9027	*E. coli* ATCC 25922	*S. Typhimurium* ATCC 14028
AQ 100%	++	+	+
AQ 75%	+	+	-
AQ 50%	+	+	-
AQ 25%	+	-	-
Gentamicin	++	+	+
DMSO	-	-	-

Conclusion

The desert is a biological environment abundant in natural resources and has significant therapeutic effects. Studies on the evaluation of natural products of animal origin are critical because this area is currently underexplored compared to plant materials, which could lead to substantial medicinal advances. According to the findings of our current study, *Chamaeleo chamaeleon* aqueous extract contains several minerals, peptides, and vitamins, giving it substantial medical significance in treating infectious and inflammatory diseases. This is what contributes primarily to the treatment of tonsillitis. Given the importance and novelty of this topic, it is interesting to continue to search for more accurate results with other types of solvents to detect active compounds in *C.Chamaeleon* powder.

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