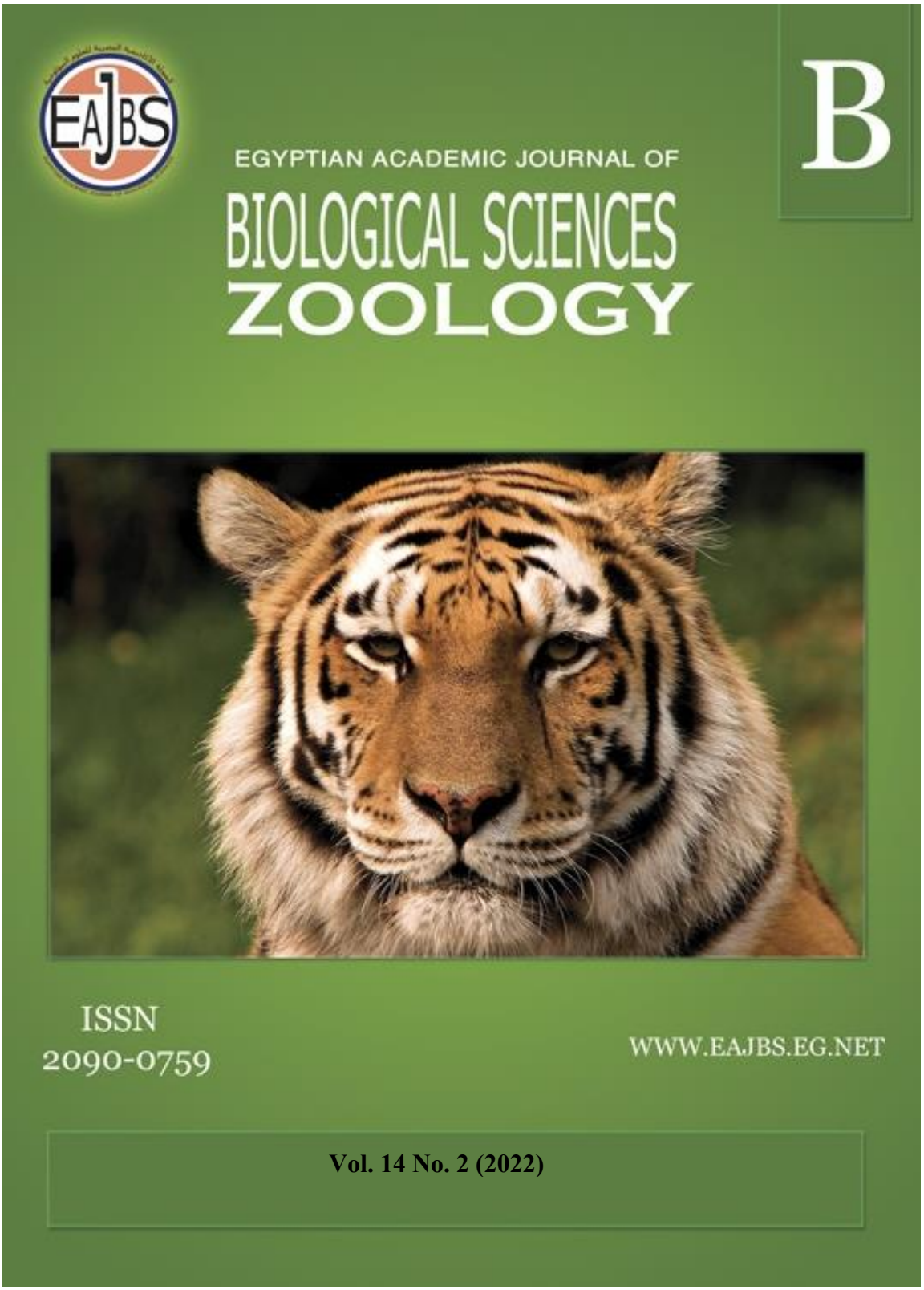

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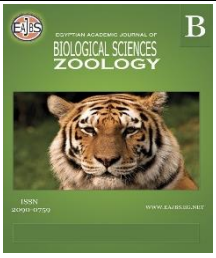

Evaluation of The Analgesic and Anti-Inflammatory Activity of The Aqueous Extract of *Anvillea radiata* from South West Algeria

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ABSTRACT

Anvillea radiata is used in traditional medicine for the treatment of chest colds, indigestion, and gastrointestinal disorders. Our plant has hypoglycaemic, antitumor, anti-inflammatory, and antibacterial activity and is used for the treatment of dysentery.

We have investigated the analgesic and anti-inflammatory activity of the aqueous extract of *Anvillea radiata*. In analgesic activity, we have used mice of the strain NMRI Albinos, weighing between 26 and 38 g (in vivo study). Acetic acid was used to induce writhes in mice, and the evaluation of the analgesic activity shows that the aqueous extract at 150 mg/kg of our plant induces a decrease in the number of abdominal cramps caused by 1% acetic acid. The aqueous extract of our plant has an analgesic effect almost equal to that of Diclofenac; in fact, the latter causes a pain inhibition of 30.28 1.1% and that of *Anvillea radiata* of 28.71 2.1% for a concentration of 150 mg/ kg. Our results of this research indicated that *Anvillea radiata* inhibited inflammation, which could explain its use in traditional medicine.

INTRODUCTION

Anvillea radiata generally inhabits small sandy-clay depressions (TELLI, A. 2017), which are distributed in the steppes of North Africa, particularly in Morocco and Algeria (Mebarki *et al.*, 2013), especially in the regions of Ouargla (Oueld El Hadj, *et al.*, 2003), Béchar (Djellouli, *et al.*, 2013), and El Golia (El Hassani, *et al.*, 2004).

Anvillea radiata is used in traditional medicine for the treatment of pulmonary chills, indigestion, and gastric-intestinal disorders (Oueld El Hadj *et al.*, 2003) and has been reported to have hypoglycemic activity and be used for the treatment of dysentery

(El Hassani *et al.*, 2004). It has been reported to have anticancer activity (Essam et al., 1996) and has also been used against the female genital tract microbe (C.R.S.T.R. A). Treatment of infectious, gastro-intestinal, dermatological, and rheumatic diseases (Moumou *et al.*, 2014).

In literature, the plant *Anvillea radiata* has been reported to contain mainly germacranolide compounds such as 9-hydroxy parthenolide and 8-hydroxy-9-epoxy parthenolide, isolated in chloroform extracts, and a less abundant fraction of phenolics (dicaffeoylquinic acid derivatives), including flavonoids (glycosides and aglycones) in aqueous and alcoholic extracts (Moumou *et al.*, 2014 ; El Hassani *et al.*, 2004 ; Kandouli *et al.*, 2017) in aqueIn addition to these active compounds, saponins, tannins, fatty acids, and various types of secondary metabolites, including sterols, terpenoids, etc., have been found in extracts of *Anvillea radiata* (Mebarki *et al.*, 2013). Many of them have biological and antioxidant activities. Germacranolides from *Anvillea radiata* have been shown to have antitumor, anti-inflammatory, and antibacterial properties (Moumou *et al.*, 2014).

MATERIALS AND METHODS

1.a. Plant Material:

The plant was collected in January 2020 in the region of Bechar (southwest Algeria). The species was identified taxonomically by Prof. Maarouf. All aerial parts were used (flowers, stems, and leaves); the plant was harvested in places where there is no chemical fertilizer; dried in the shade in a dry place away from the light; then broken and cut into small pieces to prepare the infusion.

1.b. Model Animal:

Approval was obtained from the Animal Experimental Use Committee (Approval # 943-19).

The study was conducted on male NMRI albino mice, aged 4–6 weeks and weighing 26–38 g (100% male), from the laboratory of the Algerian Pasteur Institute (NMRI strain for Naval Medical Research Institute). These mice were reared at an ambient temperature of 25±2°C with a 12-h light/dark cycle and free access to water and food. Standard food (Bovin fattening) was purchased. Before each experiment, mice were given an empty stomach for 17 hours.

Preparation of the Extract:

The aqueous extract was prepared by Soxhlet extraction of 50 grams of powder in 300 ml of distilled water for 2 hours. After decantation, the supernatant is filtered, and the filtrate is evaporated under a vacum in a rotary evaporator. A concentration range of aqueous extract of 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 750 mg/kg, and 1000 mg/kg was prepared.

Reagent Uses:

Aqueous extracts of *Anvillea radiata*, 1% acetic acid, and diclofenac sodium, 25 mg/kg

Toxicity Test:

We worked on Sephan batches of 6 mice for the aqueous extract of *Anvillea radiata*.Doses administered: 50, 100, 150, 200, 750, and 1000 mg/kg of the aqueous extract were taken with the physiological solution at 10 l/g of the mouse's body weight, and the control batch was administered with physiological water at 10 l/g.

The mice were dosed with the aqueous extract and physiological water, respectively, intraperitoneally according to their body weight. They were then observed for 2 hours in order to record immediate signs and behaviors after intoxication and to

report them to the control group. After the 2 hours, they were given food and water and then had two observation periods, one of 24 hours and one of 48 hours.

Statistical Analysis:

The obtained results were subjected to an analysis of variance (ANOVA) at a probability level of P 0.05 and the Student's test, and (n) represents the number of mice in each group.

RESULTS AND DISCUSSION

Toxicity:

Immediate signs of change, intoxication, and sudden death compared to control animals were monitored continuously for 2 h after intraperitoneal administration of the aqueous extract. After 24 h and 48 h, we also observed the mice again to count the deaths and determine the delayed effects of taking different doses of the aqueous extract of *Anvillea radiata*. Intraperitoneal administration resulted in severe changes in the physical activity and behavior of the mice, which could lead to death. (Table 1) summarizes the immediate signs recorded in this experiment.

Table 1: Results of the intraperitoneal toxicity test of the aqueous extract of Anvillea radiata.

Product	**Doses mg/kg**	**symptoms**			**mortality**
		Hypoactivity	somnolence	tachycardia	
Aqueous extract	50	-	-	-	0
	100	-	-	-	0
	150	-	-	-	0
	200	-	-	-	0
	750	++	++	++	0
	1000	+++	+++	+++	0
:+++ Very high reaction:++ Medium reaction:+ Low reaction:- No reaction.					

In this work, it is very important to note that animals given the 750 and 1000 mg/kg doses showed strong signs of hypoactivity, drowsiness, and tachycardia but showed no mortality for 2 h, 24 h, or 48 h.

Writhing Test:

The physiological water control group showed, after intraperitoneal injection of 1% acetic acid, mean abdominal contractions of (85.33±2.3) with 0% inhibition over 20 minutes (Table 2).

Subcutaneous administration of Diclofenac sodium 20 g prevented the occurrence of abdominal contractions related to acetic acid administration (59.33±1.3) with a percentage of inhibition of 30.46 percent (Table 2 and Fig. 1).

Intraperitoneal administration of an aqueous extract of Anvillea radiata in a dose-dependent manner induced the appearance of abdominal contractions in mice. With doses of 100, 150, and 200 mg/kg of the aqueous extract, the abdominal contractions observed were significantly different from those observed in the control group (71.83±5.8, 60.83±5.8 and 66.00±00) with a percentage of inhibition of 15.82%, 28.71%, and 22.65%, respectively. (Table 2 and Fig. 2**).**

At 150 mg/kg intraperitoneally, the aqueous extract of Anvillea radiata prevents the onset of pain in a manner broadly convergent with Diclofenac sodium 20 g by

subcutaneous administration. The observed abdominal contractions are (60.83±5.8) with a percentage of inhibition of 28.71%.

Table 2: Anti-inflammatory effect of aqueous extract of *Anvillea radiata* on abdominal contractions induced in mice by acetic acid injection.

Organs and doses (mg/kg)		number of abdominal contractions	Percentage inhibition (%)
witness		85.33±2.3	-
Diclofenac 20µl		59.33±1.3	30.46
extract	100	71.83±5.8	15.82
	150	60.83±5.8	28.71
	200	66.00±00	22.65

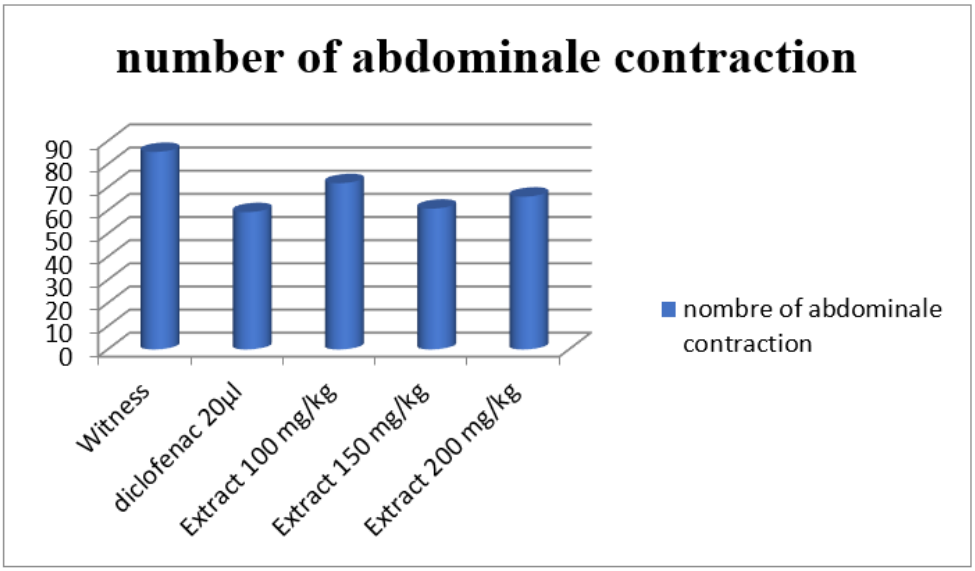

Fig. 1: Effects of aqueous extract of *Anvillea radiata* on pain induced by 1% acetic acid.

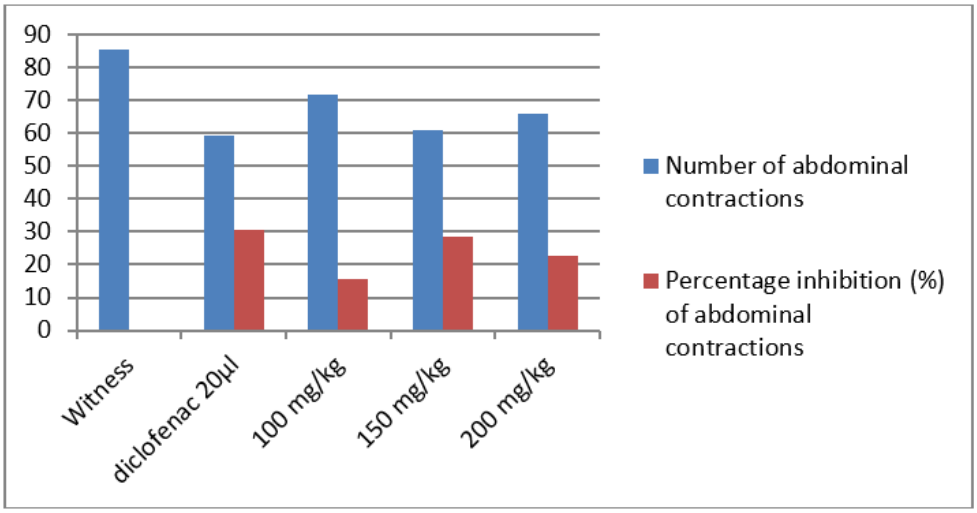

Fig. 2: Effects of aqueous extract of *Anvillea radiata* on pain induced by 1% acetic acid. Each value represents the mean and percentage inhibition of abdominal contractions.

Anti-inflammatory Activity:

The anti-inflammatory effect of *Anvillea radiata* extract was evaluated in vitro against bovine serum albumin denaturation; the results are summarized in Table 4 and Table 5.

Table 4: Results of the anti-inflammatory activity of the aqueous extract in vitro by the protein denaturation method (BSA).

Concentration (mg/ml)	Optical density (nm)	Inhibition percentage (%)
0.0012	0.162 ± 0.005	33
0.0025	0.234 ± 0.004	54
0.005	0.322 ± 0.02	66
0.01	0.461± 0.04	76
0.02	0.967 ± 0.02	88
0.04	1.387 ± 0.05	92

Table 5: Effect of diclofenac on in vitro anti-inflammatory activity by the bovine serum protein denaturation method.

Concentration (mg/ml)	Optical density (nm)	Inhibition percentage (%)
0.0012	0.151 ± 0.009	29
0.0025	0.170 ± 0.002	37
0.005	0.179 ± 0.02	42
0.01	0.291 ± 0.01	63
0.02	0.447 ± 0.009	76
0.04	0.603 ± 0.1	82

With the results presented in the tables, we can analyze and interpret the results as follows:

It can be seen that the inhibition rate of the aqueous extract of Anvillea radiata is much higher than that of diclofenac.

It can be seen that at the concentration of 0.0012 mg/ml, the inhibition rate of diclofenac is 29%, whereas the aqueous extract of Anvillea radiata shows a percentage of 33.3% (% inhibition of our extract is higher than that of diclofenac).

It is noted that the percentage of inhibition of diclofenac (37%) is much lower than that of the aqueous extract of Anvillea radiata (54%), at a concentration of 0.0025 mg/mL.It is also noted that the aqueous extract of Anvillea radiata achieved an inhibition rate of 66% at a concentration of 0.005 mg/mL, whereas diclofenac only achieved 42%.
It should be noted that the inhibition rate of the aqueous extract of *Anvillea radiata* reached 92% at a concentration of 0.04 mg/mL, unlike that of diclofenac, which did not exceed 82%. Throughout our research, we found that the higher the concentration, the higher the inhibition rate.

We concluded that the aqueous extract of *Anvillea radiata* has a high anti-inflammatory activity compared to diclofenac. Therefore, we conclude that the aqueous extract has good anti-inflammatory activity.

These results may be due to the richness of our plant's secondary metabolism.
The study of the anti-inflammatory activity of our plant has shown that it has a very high effect against inflammation by denaturing proteins. The mechanism of denaturation may involve a alteration of electrostatic, hydrogen, hydrophobic, and disulfide bonding. This

may be due to the chemical compounds that make up the plant *Anvillea radiata*. This is confirmed by Kar *et al.* (2012): protein denaturation is one of the causes of inflammation. The production of autoantigens in inflammatory diseases may be due to in vivo denaturation of proteins. Mahdjar *et al.* (2020) show conclusively that Anvillea radiata extract has good anti-inflammatory activity in vitro. This could be due to the presence of secondary metabolites contained in this plant that have interesting biological activities (antibacterial, antifungal, anti-inflammatory, anti-cancer, antioxidant, etc.). On the other hand, according to Roberts *et al.* (2020), the plant proved its effectiveness in the direction of biological activity (anti-inflammatory and antimicrobial) due to the chemical compounds that constitute the plant Anvillea radiata. The aqueous extract shows significant anti-inflammatory activity. Evaluation of the anti-inflammatory activity of the aqueous extract shows that this plant has pharmacological potency, which supports its rational traditional use for the relief of various inflammatory conditions. This confirms that the Anvillea radiata plant is endowed with very high anti-inflammatory properties.

Conclusion

Medicinal plants are still the source of active principles known for their therapeutic properties. In this context, we were interested in the study of the chemical and biological composition of the aqueous extract of a Saharan medicinal plant, Anvillea radiata. The Asteraceae family was chosen on the basis of their traditional uses. The objective was to provide elements for the validation of certain properties of the plant studied, the identification of their active ingredients, and the development of methods for evaluating biological activities such as anti-inflammatory and antimicrobial activity. To do so, we first performed a preliminary screening of the different families of secondary metabolites contained in our Asteraceae species. The extract of Anvillea radiata has potent and dose-dependent anti-inflammatory activity in vitro. This is a discovery that scientifically justifies its use as an anti-inflammatory in traditional medicine. The overall results obtained constitute a scientific justification for the traditional use of the plants studied and further confirm the relevance of traditional remedies in the treatment of many ailments.

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