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Research Article

In Vitro Evaluation of Antioxidant and Anti-inflammatory Activities of β-carboline Roots of *Peganum harmala* L.

AMEL BENBOTT[1]*, SAIDA KAROUCHE[1], YASMINA MOUMEN[2], SABEH BOUKERIA[3], CAMILLIA MOSBEH[1], TAKWA BOUMARAF[2], DJIHANE AYADI[2]

[1] Laboratory of Natural Substances, Biomolecules and Biotechnological Applications, Department of Natural and Life Sciences, Larbi Ben M'Hidi University, Oum El Bouaghi, Algeria.

[2] Larbi Ben M'Hidi University, Oum El Bouaghi, Algeria.

[3] Laboratory of Natural Sciences and Materials, Mila University Center, Algeria.

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ABSTRACT

Peganum harmala L. belongs to the family Zygophyllaceae, and it is one of the most widely used plants in Algerian folk medicine in the treatment of jaundice and relieving joint pain and rheumatism. The aim of this study is to contribute to the evaluation of some biological activities of alkaloids extract of *P. harmala* roots, which are grown in Sidi El-Raghis region - Oum El Bouaghi country. Using the classical method of extraction of β-carboline alkaloids, the yield of alkaloids in the roots was estimated at 2.34%. Harmine and harmaline were the components identified in total alkaloids roots extract by using high performance liquid chromatography method. The analysis of antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) methods showed that the crude alkaloids extract of *P. harmala* had a significant activity in capturing free radicals (IC_{50}= 46.89±0.31 µg/mL), (EC_{50}=0.12±0.05 µg/mL), respectively. The results of the anti-inflammatory activity showed for the alkaloid compounds, there were significant differences compared to the control compound represented by diclofenac, the best value was recorded with the harmine compound (EC_{50}= 22.71± 0.52µg/mL). We conclude from this research, that harmala alkaloids have anti-oxidant and anti-inflammatory properties, this study is a good indication in the future development of plant-based drugs against anti-inflammatory and antioxidant activity.

INTRODUCTION

Natural products have been an essential source of medicine throughout the history of human civilization. These products originate from various living organisms such as microorganisms, plants, vertebrate and invertebrate animals, and marine organisms [1].

Plants are the main source for the creation of various natural products, on which traditional medicine is based. In developed countries, more than 80% of the population has recently relied on medicinal plants in their health care for several incurable diseases [2].

To date, 61% anti-cancer agents and approximately 49% anti-infective compounds are directly inspired from nature [3].

Nitrogenous compounds, or alkaloids, are the most important group of natural substances that belong to secondary metabolism. More than 12,000 alkaloids have been identified, present in about 20% of plant species [4].

P. harmala, commonly called Harmalin Algeria, is a plant of the Zygophyllaceae family. It is a perennial herbaceous plant, wild evergreen, smooth, without appendages or hairs, with a height ranging between (30-100) cm [5]. Its roots are a deep and creeping peg, its depth reaches 6 m [6]. Its stems are short and branched in all directions. Its leaves are simple, opposite, alternate, and elongated. Their length is from (2-5) cm, and their width ranges between (0.5-1.3) cm. Its flowers are large with a diameter of (2-3) cm long, consisting of 5 green sepals and 5 white

Author for Correspondence:
Email: malika1959@yahoo.fr

petals [7]. The fruits are globular capsule with 3 chambers that opens with six longitudinal valves, releasing numerous black, ribbed seeds [8]. The seeds contain a red dye that is used to color wool and carpets [9]. Various parts of this plant such as seeds, bark, and roots offer bioactive compounds that promote health benefits [10].

This plant grows spontaneously in steppes and semi-arid regions, and is widespread in the Mediterranean region, Australia, Central Asia, and America [11].

P. harmala is an important source of natural products, mainly β-carboline alkaloids [12]. Several pharmacological properties attributed to β-carboline alkaloids have been described in the literature [13], making it an important class of natural products [14] among which anti-cancer [15], anti-microbial [16], anti-depressant [17], anti-oxidant and anti-inflammatory [18].

Harmala alkaloids can form a dangerous interaction with antihistamines and antihypertensive [19]. Many studies have shown that harmine alkaloid inhibits the growth of different types of cancer cells, such as stomach cancer [20], lung cancer [21], and cervical cancer cells [22].

This research aims to extract β-carboline alkaloids from the roots of *P. harmala* plant growing in Eastern Algerian and evaluate their antioxidant and anti-inflammatory activities.

MATERIALS AND METHODS

Collection of the Sample

In this research study, the roots of the of *P. harmala* plant, which was collected from Mount Sidi Argis, East zone of the Oum El Boughi on September 24, 2021. Then After that, we sent the sample to the Algerian National Herbarium to determine the taxonomic classification. The roots were dried in a dry and dark place, then ground to obtain a fine powder. The powder was maintained in dark tightly closed bottles until a chemical study is carried out on it. The experimental study was held in the Laboratory of natural substances, biomolecules and biotechnological applications of Oum El Boughi in 2022.

Chemicals

Harmine (Aldrich-286044) and Harmaline (Aldrich-51330) were purchased from sigma Aldrich, while, other chemicals and reagents including TLC solvents were purchased from Merck, Darmstadt, Germany.

Extraction and Determination of β-carboline Alkaloids by High-performance Liquid Chromatography (HPLC):

The roots powder are treated in hydrochloric acid (0.1N), then the compounds are extracted with a polar solvent, the solution containing the salt alkaloids is concentrated and treated according to the protocol shown in Fig. 1.

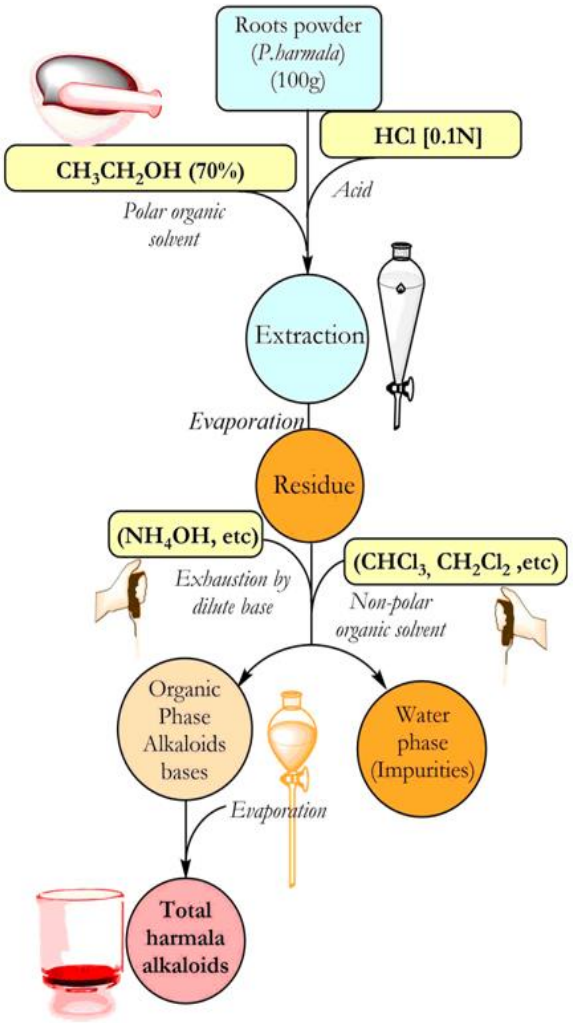

Figure 1: Extraction of harmala alkaloids in acidic medium

The extraction yield (%) was calculated as follows:

$$\text{Total alkaloids yield \%} = \frac{\text{Weight of the extract after evaporation}}{\text{plant sample weight}} \text{X100}$$

The alkaloids extracted from the roots were determined by HPLC. Details of this process have been presented in Benbott et al. [18].

Biological Activities of the Plant

Antioxidant Activity In Vitro

The antioxidant activity of alkaloids extracts (harmine, harmaline, and the crude alkaloids) of *P. harmala* has it was evaluated by adopting the free radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP).

DPPH Radical Scavenger Effect

The alkaloid extracts were tested for antioxidant potential, using the technique outlined by Benouchenne et al. [23] with some modifications. A 0.4 mM solution of DPPH in methanol was prepared, 160µL of this solution was added to 40 µl of sample diluted in methanol at different concentrations. After 30 minutes of incubation in the dark, the absorbance was measured at 517 nm, as ascorbic acid was used as a control for this study. The following equation was used to calculate the DPPH root catching ability.

$$\text{inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of the sample}}{\text{Absorbance of control}} \text{X100}$$

A lower IC_{50} value indicates higher free radical scavenging activity.

Ferric Reducing Antioxidant Power (FRAP)

Reducing power was measured by the reduction of colourless ferric complex (Fe^{3+}tripyridyltriazine) to blue-colored ferrous complex (Fe^{2+} tripyridyltriazine) by the action of electron donating antioxidants at low pH, using the method of Ozgen et al. [24] with some modifications. Wherein 0.4 mL of each extract/fraction or standard (Ascorbic acid) in methanol at different concentrations with 1 mL of phosphate buffer (0.2 M; pH = 6.6) and 1 mL of ferricyanide [$K_3Fe(CN)_6$] at 1%.

After the mixture was incubated at 50°C for 30 minutes, and then 1 mL of 10% trichloroacetic acid (CCl_3COOH) is added to stop the reaction. The mixture is centrifuged at 3000 rpm for 10 minutes for 10 min. To 1 mL of the supernatant are added 1 mL of distilled water and 0.5 mL of 0.1% iron chloride ($FeCl_3$).

The absorbance of the reaction mixture is read at 700 nm against a blank, which contains all reagents except $FeCl_3$. The reducing power of iron in the samples tested compared to the standard used is calculated according to the following formula:

Reducing power
= (Absorbance of $FeCl_3$
− Absorbance of $FeCl_3$in the presence of the extract or standard)/(Absorbance of $FeCl_3$) X100

Anti-inflammatory Activity In Vitro

The *in vitro* anti-inflammatory activity of alkaloid extracts of *P. harmala* were tested via the protein denaturation method using bovine serum albumin (BSA) [25].

In this process, 0.5 mL of different concentrations of alkaloids extract or reference compound (diclofenac sodium.), are mixed with 0.5 mL of BSA (0.2% w/v) in a Tris-HCl buffer (pH 6.8) was added, incubation at 37°C for 15 min. then in a water bath at 72°C for 5min. After cooling, the absorbance was read by the UV visible spectrophotometer at 660 nm, and the percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ I} = 1 - \frac{\text{absorbance of test sample}}{\text{absorbance of control}} \text{X100}$$

Data Processing

The analysis of the results was carried out by Microsoft® Office Excel 2010, and Microcap Origin 6.0 Professional for the graphs.

RESULTS AND DISCUSSION

Extraction of Total Alkaloids and Their Identification by HPLC

The extraction of total alkaloids from *P. harmala* roots made it possible to obtain a brick-red colored extract with a yield 2.34%. HPLC chromatography of alkaloids extract of *P. Harmala* roots (Fig. 2) recorded two peaks, which are identical to the bioactive compounds harmine (Fig. 3) and harmaline (Fig. 4), which were determined by evaluation of the retention time and fragmentation pattern.

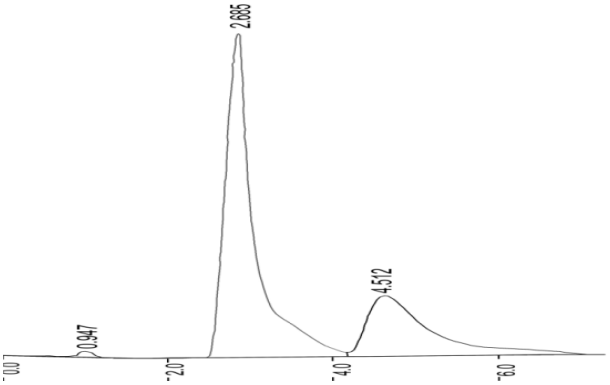

Figure 2: Chromatogram of *P.harmala* roots extract: harmine (2.685min) and harmaline (4.512 min)

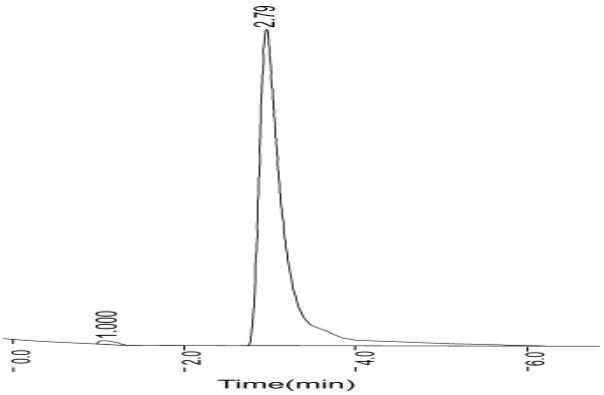

Figure 3: Chromatogram of methanolic sample of harmine (2.79min)

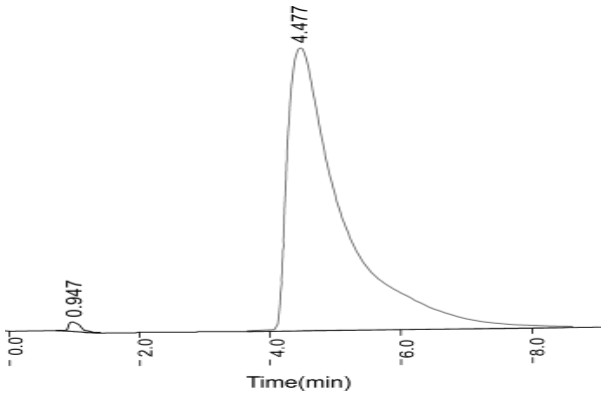

Figure 4: Chromatogram of methanolic sample of harmaline (4.47min)

Table 1: List of phytochemical compounds present in *P. harmala* root extract as recognized by HPLC analysis.

Peak No	Name of the constituent	RT (min)	Area (uv*Sec)	% Area	Heigh (uv)	% Height
1		0.947	45550	0.640	3469	1.04
2	**Harmine**	**2.685**	5037220	**70.593**	205976	83.163
3	**Harmaline**	**4.512**	2052720	**28.767**	38232	15.832
Total			7135490	100	247677	100

Identified plant compounds are included in Table 1. The chromatogram of the extract clearly showed the presence of harmine and harmaline in the roots extract, which were associated with antioxidant and anti-inflammatory activities.

DPPH Free Radical-Scavenging Activity

The tested alkaloids samples showed significant activity in scavenging DPPH radicals in a concentration-dependent manner when compared to standard ascorbic acid. The results of the experiment are reported in Fig. 5 and 6.

The greatest inhibition was recorded by the crude alkaloids extract of at a concentration of 100 µg/mL (91.04% ± 0.1), followed by the compounds of harmine and harmaline 80.6%±0.13 and 78.88%±0.2 respectively. On the other hand, the highest inhibitory ratio of ascorbic acid was recorded (91.81%±0.14) at the same concentration.

These percentages of inhibitors allowed us to calculate IC_{50} and compare the efficacy of the extracts. We remember that the lower the value of IC_{50}, the greater the strength of the extract. The three extracts demonstrates a great capacity to trap the DPPH˙ radical, with the IC_{50} values for the extracts of the crude alkaloids of *P. harmala* , harmine and harmaline were estimated to be 46.89, 47.81 and 50.83 µg/mL, respectively.

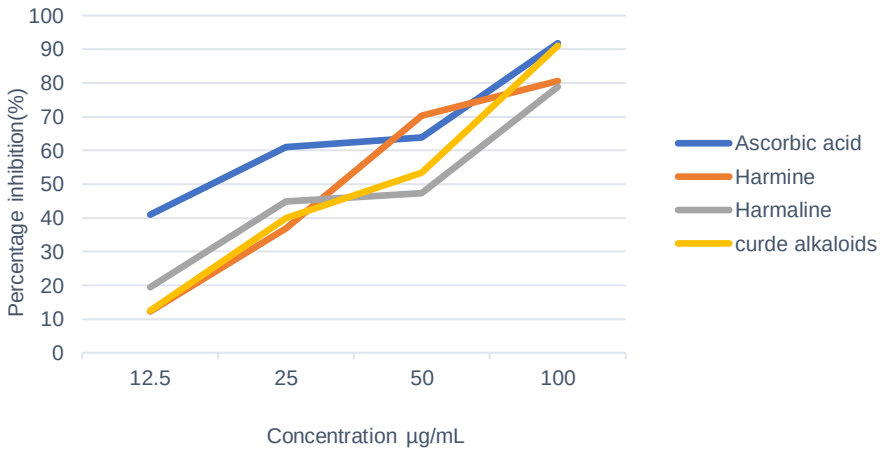

Figure 5: Inhibition of the DPPH radical by alkaloids of *P. harmala* extracts and standard

Ascorbic acid it is a reference antioxidant, has a percentage significant inhibition of DPPH radical at very low concentration. The IC $_{50}$ of standard is less than IC_{50} 19.18 µg/ mL (Fig. 6).

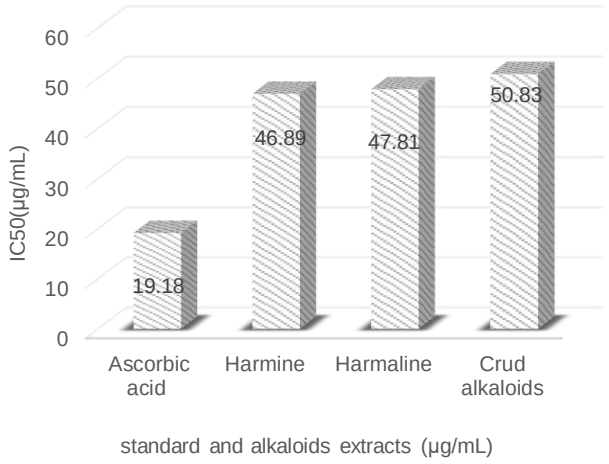

Figure 6: IC_{50} of alkaloids *P. harmala* extracts and standard

The mechanism of the reaction between the antioxidants and DPPH depends on the structural conformation of the antioxidant. DPPH radical can be influenced by the structural characteristics of the antioxidant molecule which leading to the reduction of DPPH number equal to that of the hydroxyl groups present in the antioxidant compound [26].

Several of them have been used in previous work to determine the antioxidant activity of plants [27, 28]. The results indicate that alkaloids *P. Harmala* extracts contain free radical scavenging agents that act as primary antioxidants. The effect of these antioxidants is believed to be due to their ability to donate hydrogen or electron atoms [29]. The results of Abbas et al. [18] revealed that the 100% methanol extract of *P. harmala* shows *in vitro* antioxidant activity in a DPPH assay with an IC_{50} of 49µg/mL compared to standard quercetin with an IC_{50} of 25.4µg/mL.

Ferric Reducing Antioxidant Power (FRAP)

The antioxidant activity of alkaloids *P. Harmala* extracts were evaluated using the FRAP method, which is a quick and easy-to-perform method, and this method is based on the ability of the extracts to reduce ferric iron Fe^{3+} to ferric iron Fe^{2+}. The results (Fig. 7) showed that there is a proportional relationship between increasing the concentration and reversibility of different alkaloids extracts.

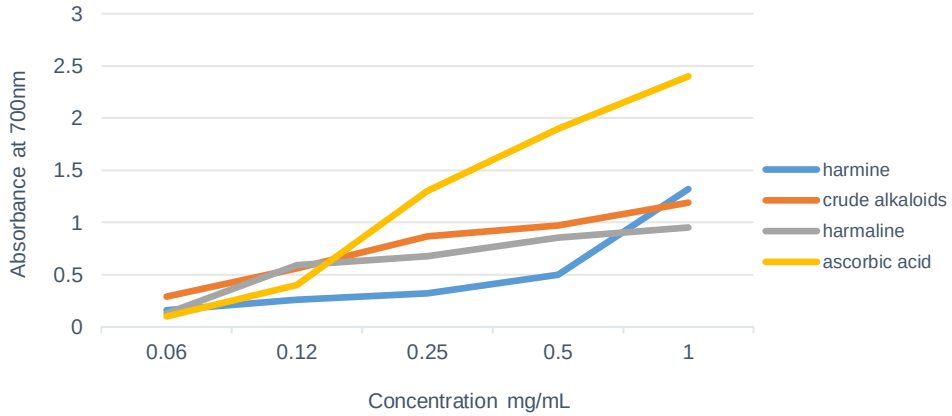

Figure 7: Reducing power by alkaloid *P. harmala* extracts and standard

The highest absorption value (2.4±0.04) was recorded at a concentration of 1 mg/mL for the reference compound ascorbic acid, while the highest absorption value was estimated for the harmine compound (1.32±0.09), followed by the crude alkaloids extract (1.19±0.11) and finally the lowest absorption value was recorded with the harmaline compound (0.952±0.18) at the same concentration.

The crude alkaloids extract and harmaline also show good activity against the FRAP reaction, with an EC_{50} values of 0.12±0.13 mg/mL and 0.175±1.17 mg/ml respectively, although less active than the positive control (0.077±0.02 µg/mL) (Fig. 8).

The results of Guergour et al. [30] conducted by the FRAP method showed that the crude seed extract of the *P.harmala* plant is the most active with a maximum optical density of 0.667 nm at a

concentration of 0. 8 mg / mL, followed by the crude extract of leaves with an absorbance of 0.304 nm, these results are lower than ours.

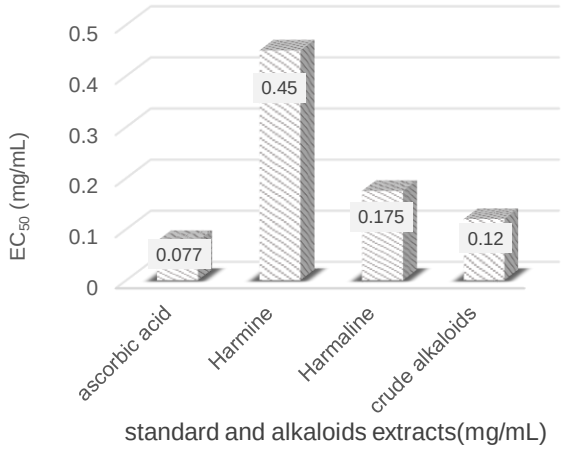

Figure 8: EC_{50} of FRAP in the presence of standard and alkaloids extracts

The difference in the results is due to the different type of chemical compounds tested in the *P.harmala* plant, as well as the difference in the studied plant organ and the quality of the extraction method.

Recently, *P. harmala* leaf methanolic extract was reported to exert antioxidant activities in DPPH (IC_{50} 21.5 µg/mL) and FRAP (IC_{50} 32.4 mM TEAC/g) assays parallel to the standard quercetin outlined IC_{50} of 21.5 µg/mL (DPPH) and 32.6 mM TEAC/g (FRAP) was also identified and quantified with a reasonable amount of phenolic compounds [31].

Anti-inflammatory Activity In Vitro

Protein denaturation is a clear cause of inflammation, and many studies have confirmed a link between inflammation problems and tissue protein denaturation [32, 33].

Our results (Fig. 9) showed at 500 µg/mL, a significant anti-inflammatory effect was obtained by the crude alkaloid extracts of roots with an inhibition percentage (98.09%), followed by harmaline (96.03%), and harmine (90.12%), these inhibition percentages are very close to the inhibition degrees of diclofenac (99.6%).

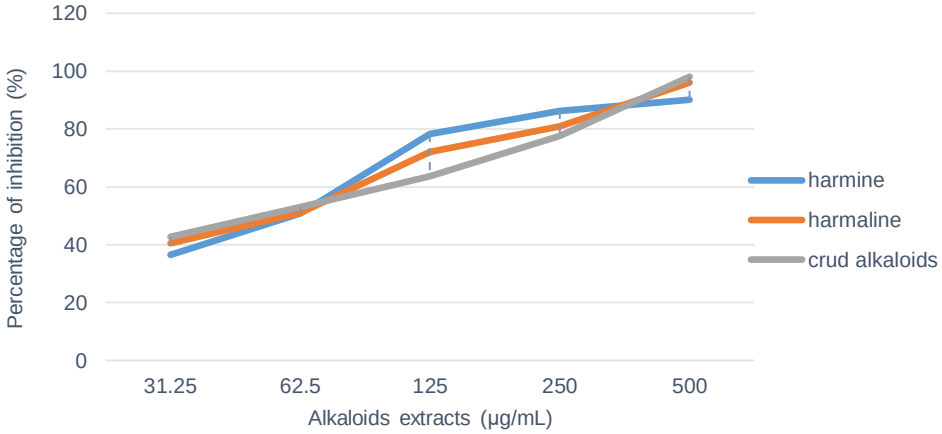

Figure 9: *In vitro* anti-inflammatory effect of alkaloid *P. harmala* extracts

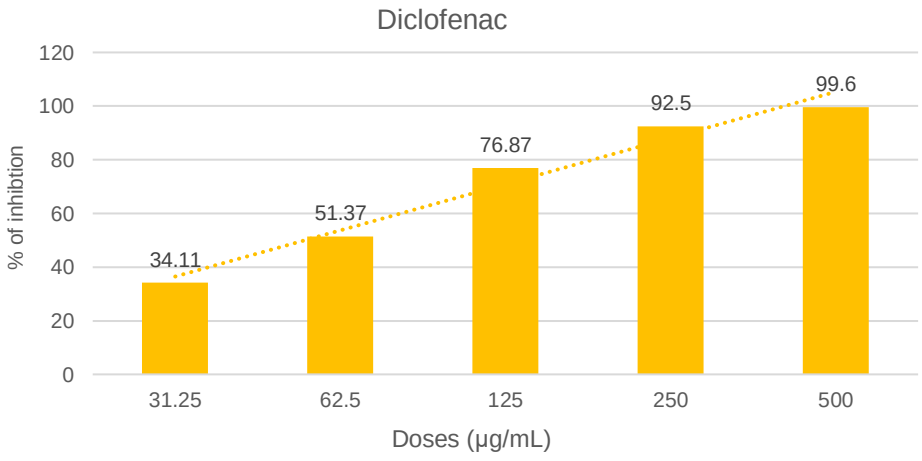

Figure 10: *In vitro* anti-inflammatory effect of diclofenac

As presented in Fig. 11, the alkaloids extracts, as compared to the diclofenac, showed a concentration-dependent inhibitory activity against the protein denaturation induced by the high temperature. The EC_{50} of the crude alkaloids extracts of *P. harmala*, harmaline and harmine (40.32, 26.51 and 22, 71µg/mL) was higher compared to the EC_{50} of the diclofenac standard (63.30µg/mL)

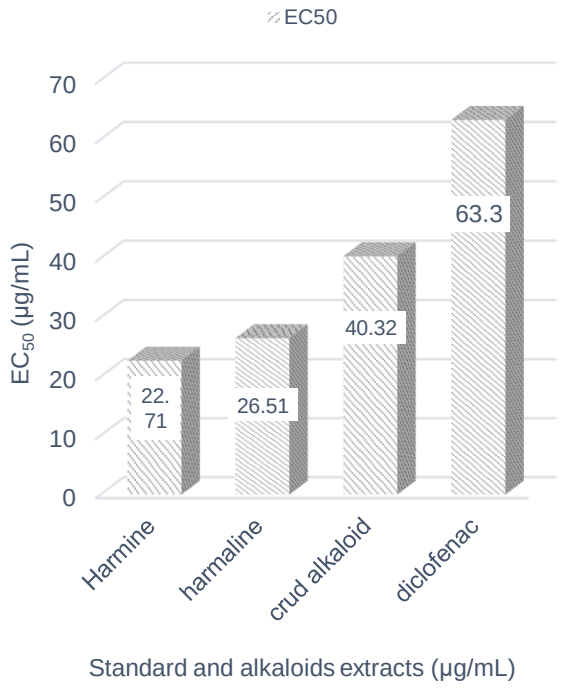

Figure 11: EC_{50} of alkaloids of *P. harmala* extracts and standard

The results of the anti-inflammatory activity *in vitro* showed that the alkaloid extracts of the roots have a high ability to preserve the three-dimensional protein structure; these findings are consistent with the results obtained by the standard diclofenac.

The results we obtained are very high compared to the study by Abbas et al. [18], who showed that the methanolic extract of the *P.harmala* plant showed 63.0% inhibition against serum albumin denaturation at a concentration of 400µg/mL; compared to 97% inhibition by the standard diclofenac sodium in an anti-inflammatory laboratory test.

The study by Akhtar et al [34] indicated that *P. harmala* possesses anti-arthritic and anti-inflammatory activity, which is due to the presence of alkaloids, phenols and flavonoids.

These results are in accord with those obtained by Lekmine et al. [35], which showed a concentration-dependent inhibition against denaturation of egg albumin by experimental *A. gombiformis* extracts and standard diclofenac sodium.

Ali et al. [36] showed in his study that the alkaloid harmine protects the kidneys from damage caused by cisplatin, as the antioxidant, anti-inflammatory and anti-apoptotic properties of harmine contributed to this therapeutic effect.

The results of Liu et al. [20] indicate that harmine succeeded in avoiding inflammatory damage to the lung significantly, and in reducing the levels of TNF-α, interleukin-1β (IL-1β) and IL-6 in the blood. This study showed that harmine may may exert an anti-inflammatory effect by inhibiting the pathway NF-κB signaling, and harmine is likely responsible for the anti-inflammatory effects of *P. harmala*.

CONCLUSIONS

The results of the present study of this research showed that the roots extract of *P. harmala* plant contains two types of alkaloids, which are harmine and harmaline.

An *in vitro* study confirmed that these alkaloids have antioxidant activity because they showed antioxidative properties in DPPH and FRAP antioxidant assay. Alkaloids extracts also possess anti-inflammatory properties and have better effects than diclofenac in the bovine serum albumin (BSA) test model, justifying their use in traditional herbal medicine in many countries including Algeria.

However, more clinical studies of this plant are needed to confirm the mechanisms of these biological activities.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest related to this article.

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