Journal of Bioresource Management

Volume 10 | Issue 2 Article 9

In Vitro Evaluation of Antibacterial and Anticoagulant Activities of Harmala Alkaloids Roots

Amel Benbott
Department of Natural and Life Sciences, Larbi Ben M'Hidi University, Oum El Bouaghi, Algeria, malika1959@yahoo.fr

Sabah Boukeria
Department of Natural and Life Sciences, Mila University Center, Algeria, boukeriasabah@gmail.com

Hakima Beldi
Department of Natural and Life Sciences, Mila University Center, Algeria, h.beldi@centre-univ-mila.dz

Kanza Kadi
Biotechnology, Water, Environment and Health Laboratory, Abbes Laghrour University, Khenchela 40004, Algeria, kadikenza79@gmail.com

Abdelouahab Yahia
Department of Natural and Life Sciences, Mila University Center, Algeria, a.yahia@centre-univ-mila.dz

Recommended Citation

Benbott, A., Boukeria, S., Beldi, H., Kadi, K., & Yahia, A. (2023). In Vitro Evaluation of Antibacterial and Anticoagulant Activities of Harmala Alkaloids Roots, *Journal of Bioresource Management, 10* (2).
ISSN: 2309-3854 online
(Received: Jan 13, 2023; Accepted: May 15, 2023; Published: Jun 30, 2023)

In Vitro Evaluation of Antibacterial and Anticoagulant Activities of Harmala Alkaloids Roots

Cover Page Footnote

On behalf of all the authors, we thank the members of the Laboratory of Natural Materials, Biomolecules and Biotechnology Applications, Oum El Bouaghi University, for their assistance in carrying out this research.

IN VITRO EVALUATION OF ANTIBACTERIAL AND ANTICOAGULANT ACTIVITIES OF HARMALA ALKALOIDS ROOTS

AMEL BENBOTT[1; 2]*, SABEH BOUKERIA[2], HAKIMA BELDI[2], KANZA KADI[3], AND ABDELOUAHAB YAHIA[2]

[1]*Department of Natural and Life Sciences, Larbi Ben M'Hidi University, Oum El Bouaghi, Algeria*
[2]*Department of Natural and Life Sciences, Mila University Center, Algeria*
[3]*Biotechnology, Water, Environment and Health Laboratory, Abbes Laghrour University, Khenchela 40004, Algeria*

*Corresponding author's email: malika1959@yahoo.fr

ABSTRACT

Peganum harmala L. is a wild herbal plant rich in active compounds, and is among the most used plants in local medicine. The purpose of this study is to contribute to the evaluation of some biological activities of alkaloids extract of *P. harmala* roots. Using the classical method of extraction of β-carboline alkaloids, the yield of alkaloids in the roots was estimated at 2.34 %. The thin layer chromatography (TLC) method identified the two components Harmine and Hrmaline in the total alkaloid extract of the roots. We evaluated the antibacterial activity of harmala alkaloids in roots against three referenced bacterial strains, and this using the agar medium diffusion method, all extracts showed significant activity against all tested bacterial strains, the largest inhibition zone diameter of 20 mm was recorded with *Staphylococcus aureus* at a concentration of 50 mg/ml of crude alkaloids. The anticoagulant activity of alkaloid extracts was also examined using the prothrombin time (PT) and partial activation thromboplastin time (APTT) tests. The clotting times obtained in normal plasma indicate that they have good activity in both coagulation pathways compared with sodium heparin, especially in the prolongation of blood clotting in the intrinsic pathway.

Keywords: *Peganum harmala*, alkaloids, TLC, antibacterial effect, anticoagulant activity.

INTRODUCTION

Medicinal plants are used to treat many diseases, and 80 % of the worldwide population still depends on herbal medicines, due to their richness in bioactive molecules. These natural molecules are involved in various fields such as cosmetics, food technology, and pharmaceuticals (Parsaeimehr et al., 2017). These bioactive compounds have aroused particular interest with their evaluations in various economic sectors**.** Algerian flora is very rich and diverse due to the diversity of the climate, the distinguished bio-geographical situation, and the extent of its spread between the Mediterranean Sea and sub-Saharan Africa (Baba Issa, 2011). Algerian flora is very rich and diverse due to its vital geographical location and climate diversity. Among these plants, we chose *P. harmala* belonging to the family Zygophyllaceae (Bournine et al., 2017). It is very rich in secondary metabolites, such as Glycosides, terpenes, phenols and alkaloids (Benbott et al., 2013). These compounds are of great importance in the field of health, there are several reports indicating the importance of the plant in the treatment of inflammation (Akhtar et al., 2022), against cancer (Liu et al., 2022), anti-diabetic and hypolipidemic activities (Komeili et al., 2016), anti-microbial (Wang et al., 2022), anti-oxidant (Abbas et al., 2022). Most of these biological activities result from the action of β-carboline alkaloids, which are harmine, harmaline and harmalol (Moloudizargari et al., 2013), amounting to 2-7 % total alkaloids (Benbott et al., 2013).

The aim of this work is to extract alkaloids from roots and identify their type using TLC technique, along with studying their antibacterial and anticoagulant activity. Although there are many research projects focusing on the anticoagulant activity of different plant extracts, this activity has not been studied for alkaloids extracts from *P.harmala*. Therefore, the subject of this research is the first of its kind, which falls within the framework of studies interested in valuation of harmala alkaloids.

MATERIALS AND METHODS

Collection of the Sample

The roots of the *P. harmala* plant are the subject of our phytochemical and biological studies. It was harvested from the region of Sidi Raghis, Oum El Bouaghi Governorate, in September 2022. The botanical identity of the plant was confirmed by Pr. Youcef Halis. The roots were air-dried at room temperature, then crushed and kept in a dark bottle until chemically studied.

Chemicals

The two reagents Harmine (Aldrich-286044) and Harmaline (Aldrich-51330) were purchased from sigma Aldrich, while the other chemicals and reagents, including TLC solvents and 60F254 pre-coated silica gel TLC plates, were purchased from Merck, Darmstadt, Germany

Alkaloids Extraction

100 g of roots powder are treated in hydrochloric acid (0.1 N), then the compounds are extracted with a polar solvent, the solution containing the salt alkaloids is concentrated and then treated with ammonia NH_3 (0.1 N) to obtain an extract of pH = 10, then adding 40ml of chloroform. The operation is repeated 3 times then evaporates (Benbott et al., 2013). The extraction yield (%) was calculated as follows:

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

QualitativeAanalysis of the Extracted Alkaloids by Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) study was performed using different solvent systems, and finally, it was found that methanol/chloroform/ammonia (79:20:1, v/v) was the most suitable mobile phase for good separation of the crude alkaloid extracts. The plates were detected under ultraviolet light of 365 nm and 254 nm. Dragendorf reagent was followed by heating for 5-10 minutes at 105°C. After development (the spots appeared after 10 min.). The quality of the compounds present in the extracts was determined by comparing their retention factor values with the standard alkaloids retention factor values. In a chromatogram, the mobility of components was determined by the Rf value. It is calculated as the ratio between the distance traveled by the solute (component) and the distance traveled by the mobile phase front (Rabel and Sherma, 2012). Rf value was determined by following ratio:

$$Rf = \frac{\text{distance moved by the solute}}{\text{distance moved by the mobile phase front}}$$

In Vitro Evaluation of the Antibacterial Activity of Alkaloid Extracts

The antibacterial effect of both crud alkaloids extract of *P. harmala* roots and standard alkaloids was evaluated using disc diffusion methods on solid medium. The antibacterial activity of both crud alkaloids extract of *P. harmala* roots and standard alkaloids was evaluated using disc diffusion methods on solid medium.

i. Sources of Bacterial Strains and their Activation

The reference bacterial strains tested were obtained from the Pasteur Institute, Algiers, these strains are identified in Table 1.

Table 1: Microbial strains tested and their American Type Culture Collection (ATCC)

Bacterial strains	Code	Gram
Staphylococcus aureus	ATTC 25923	Positive
Escherichia coli	ATCC 25922	Negative
Pseudomonas aeruginosa	ATCC 27853	Negative

The reactivation of the preserved bacterial strains is a necessary step before using them in order to obtain a young and pure culture. Where the strains are inoculated in a nutritious broth suitable for their growth, then they are incubated at room temperature for 24 hours, so that a density of a bacterial suspension of 108 colony forming units per milliliter (cfu/ml) is obtained.

ii. Preparing Disks

The alkaloid extracts from the roots were dissolved in DMSO solution, and 6 mm diameter discs were soaked with 10 µL of different concentrations for each extract, and control discs were soaked only with pure DMSO (10 µL).

iii. Diffusion Method on Agar Medium

The method of diffusion on agar medium was described by Espinel-Ingroff et al., (2011), this process consists in dipping a sterile swab into the bacterial suspension, Next, rubbed the swab over the entire agar surface, from top to bottom, in narrow strips, while rotating the disk 60° each time. Whatman paper discs saturated with different concentrations of alkaloids extracts were then placed in petri dishes, repeating the experiment three times for each extract and for each bacteria to reduce experimental errors. The dishes were incubated at room temperature for 18 to 24 hours. The measurement was performed by measuring the diameters of the inhibitory zones in millimetres.

Anticoagulant Activity

This activity was assessed in vitro by according to the process described by Athukorala et al., (2007) that relies on two clotting pathways (the intrinsic pathway and the extrinsic pathway). This effect was on a pool of depleted normal plasma and using twochronometric tests, the prothrombin time assays (PT) and activated partial thromboplastin time (APTT).

i. Endogenous Coagulation Pathway (APTT)

100 µL of plasma was mixed with different concentration of Alkaloids extracts (50, 25, 12.5 µg/mL) prepared at a certain concentration, then incubated at 37 °C for 15 minutes, 100 µL of Cephalin-Kaulinin was added, the solutions were incubated for 3 minutes with stirring at 37 °C. Then the clotting time was determined using a coagulometer by adding 100 µL of preheated calcium chloride (0.025 M). Under the same conditions, a positive control test for heparin (0.01 mg/mL) and a negative control test for sodium chloride solution (0.9 %) are performed, the results expressed by clotting per second (s).

ii. Exogenous Coagulation Pathway (PT)

The effect of alkaloid extracts on the exogenous coagulation pathway was tested following the model described by Wing et al., (2010). The factors are activated and the time of clot formation is measured. 100 µl of platelet-poor plasma preheated for 2 min at 37 C° is mixed with different

concentration of extracts (50, 25, 12.5 µg/mL). After 15 min of incubation at 37 °C, 200 µl of calcium thromboplastin (preheated for at least 15 minutes at 37 °C) is added to the mixture and the coagulation time is then recorded using a coagulometer.

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 TLC

The total alkaloids extraction yield from *P. harmala* roots was estimated to be 2.34 %. TLC chromatography of alkaloids extract of *P. harmala* roots recorded two spots, and this is after showing it by the Dragendorrf reagent that reacts with the alkaloids giving a spots orange color. This spots were observed at 365 nm under UV light were fluorescent bluish green and violet stains. This compounds which corresponds to the harmaline and harmine standers, which were determined by evaluating the factor Retention (R*f*) at 0.30 and 0.71, respectively (Figure 1 and Figure 2) and table 2.

The obtained results are consistent with the research carried out by Saad et al., (2019)., which showed, through TLC chromatography, that the seeds of the *P. harmala* plant growing in the Baghdad region contain two types of alkaloids with a retention factor equal to 0.29 and 0.79, and this is done by using a solvent system consisting of methanol / chloroform (50: 50), and in another study, Benbott et al.(2022), showed that the seeds of the *P. harmala* contain β-carboline alkaloids represented in harmine, harmaline, and harmol, which are the most common types of alkaloids present in it.

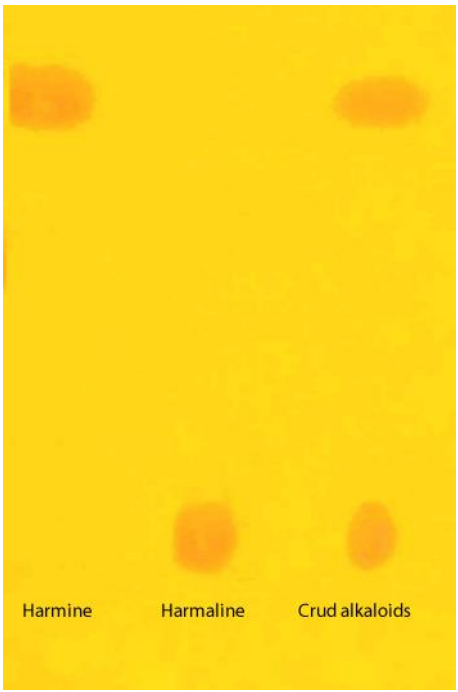


Figure 1: TLC for standards harmaline, harmine, and crud alkaloids detection with Dragendorff reagent.

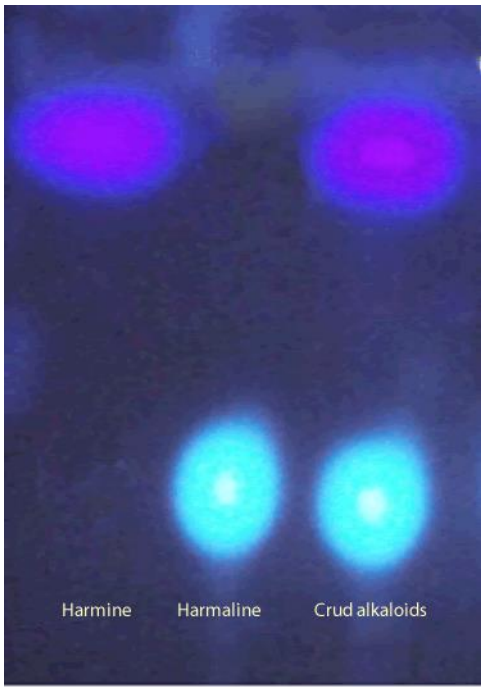


Figure 2: TLC for standards harmaline, harmine, and crud alkaloids respectively from left to right under UV light.

Table.2: TLC of crud alkaloid extraction compared to standards (harmine and harmaline).

solvent system	Spot number	Spot type	Retention factor	Color under UV_{365}	Color with Dragendroff agent
methanol/chloroform/ammonia (79:20:1, v/v)	1	Harmine	0.71	Violet	Orange
	2	Harmaline	0.30	Bluish green	Orange
	3	Crud alkaloids	0.71 0.30	Violet Bluish green	Orange Orange

Table 3: Antibacterial activity of crud alkaloid extraction compared to standards (harmine and harmaline)

Bacterial strains	Diameters of inhibition zone diameter (mm)												
	Harmine (mg/ml)				Harmaline (mg/ml)				Crude alkaloids (mg/ml)				DMSO
	50	25	12.5	6.25	50	25	12.5	6.25	50	25	12.5	6.25	
S. aureus	19	17	14	10	16	13	11	9	**20**	18	15	11	-
E. coli	14	11	10	9	13	11	10	9	17	15	12	10	-
P. aeruginosa	9	8	7.5	7	8.5	7	7	6	8.5	8	7	6	-

Antibacterial Activity

The antibacterial activity of Haramla alkaloids was evaluated against three reference bacterial strains at four concentrations (50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml), using the agar diffusion method. The inhibition zones of the alkaloid extract were calculated. The results of the zones of inhibition assay are shown in Table 3.

Table 2 and Figures 3, 4 and 5 show the effect of the standards alkaloids and the crude alkaloids extracted from the roots on the growth of the tested bacterial strains. We recorded varying diameters dependent on the dose and on the type of bacterial strains. Significant values were recorded with *S. aureus* strain, where the largest diameter of inhibition was estimated to be 20 mm with the crude extract of alkaloids in the roots, followed by 19 mm with harmine alkaloid, then harmine by 16 mm at a concentration of 50 mg/ml, while the lowest inhibition zone diameter of 6 mm was estimated with *P. aeruginosa* strain at a concentration of 6.25 mg/ml.

Our results showed that the diameters of the inhibition zones ranged from 6 to 20 mm and are concurrent with varying concentration of alkaline extracts, these results agree with the previous study by Benbott et al., (2012) who found that the diameters of the inhibition zones increased with increasing concentrations of *P. harmala* extracts. In another study, Iranshahy et al., (2019) showed that alkaloids and smoke of *P. harmala* specifically are more effective on *Candida albicans* and $Gram^+$ bacteria (*Micrococcus luteus* and *Staphylococcus aureus)*, while $Gram^-$ bacteria, especially *Pseudomonas aeruginosa* were less sensitive.

Many scientific researches (Apostolico et al., 2016; Benyouneset al., 2016; Zhu et al., 2022) indicated that *P. harmala* extracts have more effect and sensitivity on $Gram^+$ bacteria than $Gram^-$ bacteria. The hypersensitivity of the *S. aureus* strain is caused by variations in the external environment such as temperature, pH and natural extracts, due to the absence of an external membrane which makes it very sensitive.

Abdulridha et al., (2019) proved in his study that *P. harmala* extracts has an effect against some pathogenic bacterial strains isolated from *Streptococcus,*

Staphylococcus, Aeromonas, E.coli, Klebsiella and *Acinetobacter*.

Anticoagulant Activity

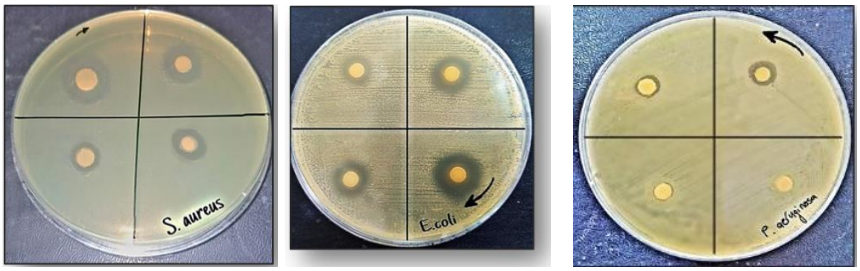
All the anticoagulant activities of alkaloids extracts from *P.harmala* roots and standards were evaluated in vitro with respect to the exogenous pathway and the endogenous pathway of coagulation using two general chronometric tests that explore coagulation APTT and PT, respectively. The results of the means of the APTT and PT tests for anticoagulant activity as a function of time are presented in Table 4.

S. aureus *E. coli* *P. aeruginosa*

Figure 3: Effect of crude alkaloids on three types of bacterial

S. aureus *E. coli* *P. aeruginosa*

Figure 4: Effect of Harmaline on three types of bacterial strains



S. aureus *E. coli* *P. aeruginosa*

Figure 5: Effect of Harmine on three types of bacterial strains

Table 4: APTT and PT of Harmala alkaloids extract and standards

Concentration (µg/mL)	Anticoagulant activity (S)									
	Harmine (µg/mL)			Harmaline (µg/mL)			Crude alkaloids (µg/mL)			Heparin (µg/mL)
	12.5	25	50	12.25	25	50	12.5	25	50	10
APPT (S)	30.77	33.38	37.9	28.39	31.9	37.70	29.19	32.731	36.56	42.18
PT (S)	12.90	16.83	23.5	13.10	17.6	23.20	13.75	16.69	23.60	13.3

The study of the anticoagulant activity on the endogenous pathway showed that harmala alkaloids, harmine and harmaline with different concentrations (50 μg/mL, 25 μg/mL and 12,5 μg/mL) had an effect similar to that of heparin. The results of the current study show that alkaloids extracts are able to prolong APTT in a significant and dose-dependent manner. However, harmine appears to exert relatively greater anticoagulant activity than other extracts of harmaline and crudes alkaloids. The concentration 12.5 μg/mL of harmine demonstrated anticoagulant activity with APTT where the duration of clot onset was estimated to be 30.77 S, corresponding to the elongation duration of 2.38 S and 1.58 S greater than that of harmaline and the crude extract respectively. Moreover, a concentration 25 μg/mL is able to exert an anticoagulant effect on the intrinsic pathway of coagulation, estimated by APTT as 33.38 S of harmine with a lengthening of 1.39 S and 0.65 S greater than harmaline, and crude alkaloids.

A concentration 50 μg/mL of harmine shows a significant anticoagulant capacity with APTT with a time of 37.94 S, corresponding to an elongation of 1.38 S greater than that of the crude extract. However, the anticoagulant activity appears to increase with increasing concentration of the extracts.

The values of a normal TP are between 12 and 14 seconds depending on the products applied. The concentration 12.5 μg/mL of harmine and Hermaline and crude of the alkaloid capable of prolonging prothrombin time (PT) of (12.90 S, 13.10 S, 13.57 S) respectively, the elongation of the crude extract is 0.67 seconds greater than that of harmine and harmaline, with little change between these two others.

However, a concentration 50 μg/mL of harmaline had an anticoagulant activity of PT estimated at 17.67 S with elongation (0.84 S and 0.74 S) compared to the harmine and the crude alkaloid extract.

Concerning, a concentration 50 μg/mL has anticoagulant effect against the extrinsic pathway of PT estimated at 23.57 s, 23.20 s and 23.60 s of the crude alkaloids, harmaline and harmine respectively, with slight changes between them. These values obtained are characterized by significant changes compared to the Heparin positive control

This result is consistent with the work of Dhahri et al., (2020) which proved that the anticoagulant power of heparin comes from disruption of the intrinsic pathway of coagulation enzymes by forming a complex with antithrombin III. Because the thromboplastin time is a coagulation test that detects all exogenous pathway coagulation factors. A study, Khoo et al., (2014) showed that hexacic acids and polysaccharides, as well as polyphenols extracted from *M. malabathricum*, played a role in prolonging blood clotting in the intrinsic pathway. This result is agreement with Li et al., (2017) who found that several β-carboline alkaloids such as harmine, harmaline, harmane, can play various pharmacological effects.

CONCLUSION

The results of TLC chromatography showed that the root extract of *P. harmala* contains two types of alkaloids, harmine and harmaline. An in vitro study confirmed that these alkaloids have antibacterial activity, the largest inhibition zone diameter was 20 mm with *S. aureus.* Alkaloids extracts also possess anticoagulant properties and have better effects than sodium heparin standard especially in prolonging blood clotting in the intrinsic pathway. This explains the use of *P. harmala* in traditional medicine in many countries including Algeria. Further research is needed to study the mechanism of the anticoagulant activity of the alkaloid components.

AUTHORS CONTRIBUTION

AB designed the study, extracted the data and wrote the study manuscript. SB and HB contributed data analysis and interpretation of the manuscript. KK and AY read the manuscript and participated in preparing the final version of the manuscript. All authors read and approved the final manuscript.

FUNDING

This research received no external funding.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abbas MW, Hussain M, Qamar M, Ali S, Shafiq Z, Wilairatana P, Mubarak MS (2021). Antioxidant and Anti-Inflammatory Effects of Peganum harmala extracts: An In Vitro and In Vivo Study. Molecules., 26(19): 6084.

Abdulridha M M, Abdulhussein HS, Alyaseen F F, Hassan BA (2019). Phytochemical and antebacterial activity of the Peganum harmala seeds and its alkaloids. Plant Archives., 19 (1): 1439-1444.

Akhtar MF, Raza SA, Saleem A, Hamid I, Ashraf Baig MMF, Sharif A, Sohail K, Javaid Z, Saleem U (2022). Appraisal of Anti-Arthritic and Anti-Inflammatory Potential of Folkloric Medicinal Plant Peganum Harmala. Endocrine, Metabolic & Immune Disorders. DrugTargets., 22(1):49-63.

Apostolico I, Aliberti L, Caputo L, De Feo V, Fratianni F, Nazzaro F, Lucèia F, Khadhr M (2016). Chemical Composition, Chemical Composition, Antibacterial and Phytotoxic Activities of Peganum harmala Seed Essential Oils from Five Different Localities in Northern Africa. Molecules., 21: 1235.

Athukorala Y, Lee KW, Kim SK, Jeon YJ (2007). Anticoagulant activity of marine green and brown algae from Jeju Island in Korea. Bioresource Technology., 98: 1711–1716.

Baba-Aïssa F (2011). Encyclopedia of useful plants: Mediterranean flora (Maghreb, southern Europe) Plant substances from Africa, East and West. BEO Algiers: El Maarifa, p 471.

Benbott A, Yahia A, Belaidi A (2012). Assessment of the antibacterial activity of crude alkaloid extracted from the seeds and roots of the plant Peganum. harmala L. Journal of Natural Product and Plant Resources., 2(5):568-573.

Benbott A, Bahri L, Boubendir A, Yahia A (2013). Study of the chemical components of Peganum harmala and evaluation of acute toxicity of alkaloids extracted in the Wistar albino mice. J. Mater. Environ. Sci., 4 (4): 558-565.

Benbott A, Mahdi D, Zellagui A., Moumen Y, Mosbah C (2018). Effect of alkaloids extract of peganum harmala seeds on histo-function of rat's testes. J. New Technol. Mater., 8(2): 72-79.

Benbott A, Mosbeh C, Karouche S, Hamadouche N, Mahdi D (2022). Subacute hepatotoxicity of alkaloids extracts of Peganum harmala L.seeds in Wistar albino rats. NSB., 14 (2):112-120.

Benyounes BN, Dahmene T, Allouche N, Laddad A(2016) . Phytochemical, Antibacterial and Antifungal Activities of Alkaloids Extracted from Peganum harmala (Linn.) Seeds of South of Algeria. Asian

J. of Ournal of Chemistry., 26(10): 2960-2964.

Bournine L, Bensalem S, Fatmi S, Bedjou F, Mathieu V, Iguer-Ouada M, Kiss R, Duez P (2017). Evaluation of the cytotoxic and cytostatic activities of alkaloid extracts from different parts of Peganum harmala L. (Zygophyllaceae). European Journal of Integrative Medicine., 9: 91–96.

Dhahri M, Sioud S, Dridi R, Hassine M, Boughattas N A, Almulhim F, Talla ZAl, Jaremko M, Emwas AHM (2020). Extraction، Characterization, and Anticoagulant Activity of a Sulfated Polysaccharide from Bursatella leachii Viscera. ACS Omega., 5 (24): 14786-14795.

Espinel-Ingroff A, Canton E, Fothergill A (2011). Quality control guidelines for amphotericin B, itraconazole, posaconazole, and Voriconazole disk diffusion susceptibility tests with nonsupplemented Mueller–Hinton Agar (CLSI M51-A document) for nondermatophyte Filamentous Fungi. J. Clin. Microbiol.,; 49:2568–2571.

Iranshahy M, Fazly Bazzaz S, Haririzadeh G, Abootorabi BZ, Mohamadi AM, Khashyarmanesh Z(2019). Chemical composition and antibacterial properties of Peganum harmala L. Avicenna J. Phytomed., 9: 530–537.

Khoo LT, Abas F, Abdullah JO, Tohit ERM, Hamid M(2014). Anticoagulant Activity of Polyphenolic-Polysaccharides Isolated from Melastoma malabathricum L. Evidence-Based Complementary and Alternative Medicine. Article ID 614273.

Komeili G, Hashemi M, Bameri-Niafar M (2016). Evaluation of Antidiabetic and Antihyperlipidemic of Effect Peganum harmala Seeds in Diabetic Rats. Cholesterol., 1: 1-6.

Li C, Wang Y, Wang C, Yi X, Li M, He X(2017) . Anticancer activities of harmine by inducing a pro-death autophagy and apoptosis in human gastric cancer cells. Phytomedicine., 28: 10-18.

Liu C, Gao J, Liang Y (2022). Phytochemistry, pharmacology and toxicology of Peganum harmala. Bangladesh J Pharmacology., 17: 124-140.

Moloudizargari M, Mikaili P, Aghajanshakeri S, Asghari MH, Shayegh,J(2013).Pharmacological and therapeutic effects of Peganum harmala and its main alkaloids. Pharmacogn Rev., 7(14):199-212.

Parsaeimehr A, Martinez-Chapa SO, Parra-SaldívarR(2017). Medicinal Plants Versus Skin Disorders: A Survey From Ancient to Modern Herbalism.Clinical Microbiology: Diagnosis, Treatments and Prophylaxis of Infections., 2: 205-221.

Rabel F, Sherma J(2012). Stationary Phases for Modern Thin-Layer Chromatography. LCGC ., 25(6): 300–313.

Saad ZA, Mohammad MH, Al-mzaien A K (2019). Qualitative and quantitative analysis of alkaloid component in seeds from Peganum harmala L. extracts . Iraqi Journal of Cancer and Medical Genetics., 12(1): 45-52.

Wang N, An J, Zhang Z, Liu Y, Fang J, Yang Z (2022). The Antimicrobial Activity and Characterization of Bioactive Compounds in Peganum harmala L. Based on HPLC and HS-SPME-GC-MS. Front. Microbiol., 19(13): 1-12.

Wink M (2010).Annual Plant Reviews, Biochemistry of Plant Secondary

Metabolism., (2nd Ed., Volume 40, 2nd Edition) A John Wiley & Sons, Ltd., Publication.

Zhu Z, Zhao S, Wang C (2022). Antibacterial, Antifungal, Antiviral, Peganum harmala and Its Ingredients: and Antiparasitic Activities of A Review. Molecules., 27, 4161.