Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

In Vitro and In Vivo Antifungal Activity of Albizia lebbeck L.Ethanolic Extract against Keratinophilic Fungi and Its Genoprotective Potential Using Comet Assay

Abeer M. Hussain [1]
Email : **Abeer.m@csw.uobaghdad.edu.iq** [1]
[1] Deptartment Biology / College of Science for Women/ University of Baghdad/ Baghdad Iraq

Abstract

Keratinophilic fungi are one of the most significant skin diseases affecting both humans and animals. Despite the availability of numerous treatments against these fungi, their symptoms and the long duration of treatment may prevent complete recovery in a short period of time. Therefore, there is a request to search for plants from nature that can be used as alternatives to antibiotics and chemical treatments. The results demonstrated the inhibition of three types of plant extracts (petroleum ether, chloroform, and ethanol), showing that these extracts have the ability to inhibit keratinophilic fungi (Trichophyton Schoenleinii, Microsporum canis, and Aspergillus flavus) on Mueller-Hinton agar at the four concentrations used in the experiment, indicating the efficiency of the plant for use in treating cutaneous diseases caused by fungi. The highest rate of inhibition was also recorded using the extract of Ethanol at concentration 4 which is the highest concentration used in the experiment. The comet assay is used as an applied analysis to measure broken DNA strands resulting from chemical and oxidative stress. It was used in this study before and after treatment to demonstrate the efficiency of the alcoholic extract in treating fungal skin infections. Regarding the experimental animals, the apparent results from the five groups taken from male mice, with each group containing 6 mice, showed that all the mice developed cutaneous lesions by scratching method ranging from mild to moderate after two weeks of exposure to the pathogen. After two weeks, the animals were treated with an ethanolic extract from the Albizia Lebbek plant at a concentration of 4 ml/liter, which showed inhibitory efficacy against keratinophilic fungi. Using the comet assay, the extract demonstrated its efficacy and ability to not harm living cells, in addition to the therapeutic potential of the extract.

Keywords: Keratinophilic fungi, Albizia lebbeck, ethanolic extract, Mueller-Hinton agar.

الملخص

تُعدّ الفطريات الكيراتينية من أهم مسببات الأمراض الجلدية التي تُصيب الإنسان والحيوان على حدٍ سواء. ورغم توفر العديد من العلاجات لهذه الفطريات، إلا أن أعراضها وطول مدة العلاج قد يحولان دون الشفاء التام في فترة وجيزة. لذا، ثمة حاجة ماسة للبحث عن نباتات طبيعية يمكن استخدامها كبدائل للمضادات الحيوية والعلاجات الكيميائية. وقد أظهرت النتائج تثبيط ثلاثة أنواع من المستخلصات النباتية (إيثر البترول، والكلوروفورم، والإيثانول)، مما يدل على قدرة هذه المستخلصات على تثبيط الفطريات الكيراتينية**(Trichophyton Schoenleinii** ، و**Microsporum canis**، و **.(Aspergillus flavus** أظهرت نتائج اختبار نمو الفطريات على وسط مولر-هينتون، تم استخدام اربعة تركيزات في التجربة لدراسة فعالية النبات في علاج الأمراض الجلدية الفطرية. كما سُجِّل أعلى معدل تثبيط باستخدام مستخلص الإيثانول عند التركيز 4، وهو أعلى تركيز استُخدم في التجربة. يُستخدم اختبار **Comet** كتحليل تطبيقي لقياس تلف سلاسل الحمض النووي الناتج عن الإجهاد الكيميائي والتأكسدي. وقد استُخدم هذا الاختبار **(Comet)** أظهرت نتائج هذه الدراسة قبل وبعد العلاج لإثبات فعالية المستخلص الكحولي في علاج العدوى الفطرية الجلدية. اظهرت نتائج الدراسة على خلايا الحيوانات التجريبية، أظهرت النتائج من المجموعات الخمس المأخوذة من ذكور الفئران، بواقع ستة فئران في كل مجموعة، أن جميع الفئران أصيبت بآفات جلدية نتيجة الحك، تراوحت شدتها بين الخفيفة والمتوسطة، بعد أسبوعين من التعرض لمسببات الأمراض. بعد أسبوعين، تم علاج الحيوانات بمستخلص إيثانولي من نبات ألبيزيا ليبك بتركيز 4 مل/لتر، والذي أظهر فعالية مثبطة ضد الفطريات الكيراتينية. وباستخدام اختبار**comet** ، أثبت المستخلص فعاليته وقدرته على عدم إلحاق الضرر بالخلايا الحية، بالإضافة إلى إمكاناته العلاجية.

الكلمات المفتاحية: الفطريات الكيراتينية، ألبيزيا ليبك، مستخلص إيثانولي، وسط مولر-هينتون.

Introduction

Numerous compounds that are useful for human health and can be used to make pharmaceutical medications are produced by plants [1, 2]. Albizia is a significant genus that belongs to the legume family (Fabaceae), which includes about 150 different species, some of which are edible. All of its species generally include trees

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

and shrubs that grow in tropical and subtropical areas such as in Asia and Africa. Albizia (A. lebbeck) is used in alternative medicine to treat certain diseases and inflammations, as it contains many active compounds that contribute to the treatment [3]. Keratinophilic known as ringworm, which parasitize keratinous materials found in the skin such as (hands, feet, and scalp), are considered skin diseases that cause discomfort to the affected person from itching, redness, and accompanying inflammation, in addition to peeling of the skin area. Fungal infections associated with them are commonly referred to as ringworm (cutaneous and subcutaneous infections). These infections can be transmitted from one person to another through the use of personal items contaminated with the spores [4, 5]. Dermatophytes or keratinophilic fungi consume the keratinous and collagenous materials present in the skin and convert them into simple absorbable molecules through enzymes they secrete [6]. All medications that treat skin diseases (both steroidal and non-steroidal antifungals) are considered common; however, the treatment period is very long. In addition to causing side effects that persist in the patient if the infection worsens [7]. Therefore, there was a request to develop treatments from medicinal plants that have been and continue to be used to treat many diseases. The flowers, leaves, and even the roots and bark of the Albizia lebbeck L extract are used to treat joint diseases, bone fractures, asthma, and many inflammations, as it has been used by tribes living a wild life in many countries as a medicinal remedy for these diseases due to its content of many anti-inflammatory compounds such as (Oleanolic acid, Acacic acid, Melanoxetin acid, Okanin, (+) Pinitol, (-)-Leucopelargonidin, Friedelan-3-one, -γ -sitosterol and macrocyclic spermine alkaloids known as budmunchiamines L1-L6) in addition to many anti-inflammatory factors [8, 9]. The current study aims to reveal the most important compounds that help in the treatment of Keratinophilic fungi infections isolated from plant extracts and apply it to living tissues to determine its effectiveness in treating skin inflammations using the comet assay technique. [10]. The production of nuclear comets from isolated chromosomes, as well as its capability to measure DNA breaks, has been modified to detect damaged bases of the extracted DNA, by digesting the DNA after it has been broken down by an enzyme that identifies the damage and generates a break in the DNA at the site of damage (see the section on "Measuring Different Types of DNA Damage"). This technique is effective for studying oxidized bases and alkylated bases, in addition to cyclobutane dimers that are formed by ultraviolet radiation. There are key areas where the comet assay is used, such as in tests for the presence of toxic genes, to examine the toxicity or damage of new drugs, the damage of cosmetic products, or other harmful chemicals, and to uncover potential carcinogenic outcomes. This test can be performed in vivo (with analysis of various tissues from the experimental animal) or in vitro using suitable cultured cell lines. Finally, the comet assay is considered a very valuable tool in basic research for understanding mechanisms of DNA damage or DNA repair [9].

Materials and methods

1. Preparation of the Albizia lebbeck L extracts

The flowers of the Albizia lebbeck L. plant were collected in April 2024 in Baghdad, Iraq. The flowers were chopped into small pieces by crushing them using a pre-sterilized electric blender. The mashed plant material (1 kg) was sequentially extracted three times using 3 liters of (petroleum ether, chloroform, and ethanol at a ratio of 95%) at room temperature for 48 hours. The resulting liquid was then concentrated at 40° C under reduced pressure and stored in a refrigerator. The yields from the

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

petroleum ether, chloroform, and ethanol extracts were (0.33%, 1.10%, and 13.70%) respectively. Chemical tests for the active extracts were conducted using several standard phytochemical methods [11].

2. Keratinophilic fungi testing

The identification of fungi were done by [12]. Three types of Keratinophilic fungi that cause skin diseases were tested on the skin after being cultured on Sabouraud Dextrose Broth (SDB) in Petri dishes. They were later used to test the effectiveness of the extract used in the study and its ability to kill the pathogenic fungi. Different concentrations of the plant extract obtained from petroleum ether, chloroform, and ethanol were applied (0.5, 1, 2, 4 mg/L) to the plates, and then the Müller-Hinton Agar (MHA) was poured using the plate pouring method. The Keratinophilic used in the experiment were inoculated with three replicates for each concentration and each fungus. After that, the plates are placed in the incubator at a temperature of 25 ° C and examined after seven days; the radius of growth (mm) is measured and compared with the control plates that grew in the same medium without the addition of the plant extract to it [13]. The figure (1) illustrated the fungi growth.

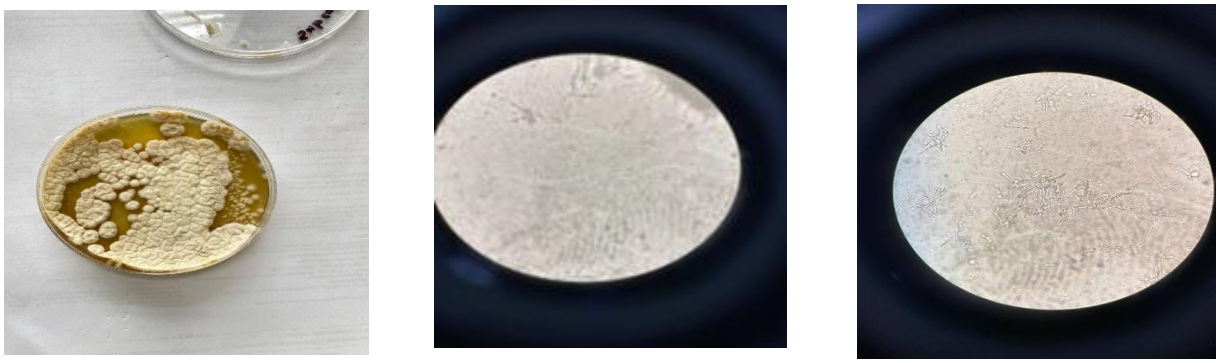


Figure (1): illustrated the fungi growth.

1. Gas chromatography–mass spectrometry (GC–MS) analysis

GC-MS analysis was performed on the raw extract of *Albizia lebbeck* using the GC-MS (5977E) instrument from the USA, which has a fused silica column. The column flow rate was maintained at a constant rate of (1.34 mL/min). The oven temperature was set from 60 to 150 degrees Celsius for 3 hours, then it was held in a thermal state for 10 minutes. The relative percentage of volatile components was expressed as ratios by normalizing the peak area. The identification of the raw extract components was based on the retention time in GC on the DB-5 column, and the computer matching of mass spectrometry methods with those of standards (Wiley 6.0 data for the GC-MS system) [14, 15].

2. Animal model

To measure and understand the effectiveness of the extract, 5 groups of mice were exposed, each group containing 6 mice, and a control group of mice was established. The mice were exposed to scratching method at a temperature of 25±2 ° C. The weight of each mouse was 25 grams. The control group was exposed to sterilized water only, without any fungal cells. The fungal cells were diluted to a concentration of inoculum (100 µl of 3×10^8 CFU/ml cells) and injected into the SDB media for a week, after which the mice were left for two weeks to allow for fungal infection. After two weeks the extract was dissolved in 2% Tween-80 in water [8, 4]. The control group was represented by the middle alone. Then, a concentration of 4 ml/liter of *Albizia lebbeck* extract was used for 5 days,

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

during which the skin of the mice was gently wiped twice a day every 12 hours using sterilized cotton. Finally, after two days, 3 ml of blood were taken from the six groups, and the blood cells (lymphocytes) were extracted for comet assay technique [16].

3. Comet assay technique

The sensitivity measurement is organized for the comet test case used in electrophoresis. This analysis has two areas: (the neutral comet test) which detects double-strand DNA breaks, while (the alkaline comet test) shows DNA breaks whether they are single or double strands. The lysis solution was prepared and cooled to 4 degrees Celsius for 20 minutes before use. In a flask containing 250 ml of boiling water, LM agarose was dissolved for 5 minutes, and then it was placed in a water bath at 37 degrees Celsius for 20 minutes. After that, the cells to be analyzed were combined at a concentration of 1 × 10^5/ml with the previously prepared dissolved LM agarose, then 50 microliters were drawn and placed directly onto the comet slide until a transparent ring with a thickness of 0.5 mm appeared at the edge of the comet slide area. After forming for 30 minutes, the samples were dried on the slide at 37 degrees Celsius for 10-15 minutes, which helps to bring all dried samples to the required level to enable observation of the results. 100 microliters of the (SYBR Green) substance were added to the dried agarose and stained for 30 minutes (room temperature) in the dark. Then, the slide was exposed to air jets to remove excess SYBR solution from agarose gel electrophoresis is washed with water and completely dried at 37 degrees Celsius to be displayed under a fluorescence microscope. (The maximum excitation/emission for SYBR Green is 496 nm/522 nm). Three thresholds were set to determine DNA migration, and the tail length is defined as the distance from the center of the head to the end of the tail), and it is calculated as follows: Number of tails produced by the DNA / Total DNA % = 100 × - DNA density in the tail / DNA density in the cell [6, 5].

Statistical Analysis

The Statistical Packages of Social Sciences -SPSS (2019) program was used to detect the effect of difference groups (patients and control) in study parameters. Least significant difference-LSD was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study [17, 18].

Results and discussions

The results showed that the extract of *Albizia lebbeck* plant is rich in active secondary compounds that have significant medicinal value, such as glycosides, steroids, alkaloids, and other compounds used as inhibitors against fungal diseases [19, 20].

The results of inhibiting, indicating the plant's efficiency for use in treating cutaneous diseases caused by fungi. Petroleum ether extract was found to have the least effect on keratinophilic fungi, with the lowest concentration used (0.5 mg/L) affecting the fungi *Trichophyton Schoenleinii*, *Microsporum canis*, and *Aspergillus flavus* with inhibition zones of 4, 3, and 3 mm respectively, at concentration 4 mg/L which is the highest concentration used in the experiment. The 4 mm concentration achieved inhibition zones of 11, 10, and 10 mm respectively. Meanwhile, the results of the chloroform extract showed good inhibition ranging at

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

the lowest concentration with inhibition zones of 2, 5, and 3 mm for the three fungal species, and at the highest concentration it reached inhibition zones of 9, 12, and 10 mm. 1The highest percentage of inhibition was recorded for the ethanol extract, where the lowest inhibition zones for the three fungal species were 7, 7, and 6 mm respectively, while the inhibition rates were the highest in the experiment, reaching 14, 15, and 13 mm as shown in Table (1). Previous studies have shown that the results obtained in this study are consistent with previous studies. The findings of researchers as Banothu, *et al*., (2017) demonstrated antifungal activity, which was also proven effective against yeast, as observed in *A. lebbeck*. The order of effectiveness was: seeds > pods > flowers > roots against certain fungal species such as *Aspergillus niger* and *Candida albicans*. Additionally, Yousif, and Hassan (2023) clarified the antifungal effects at different concentrations and explained the efficiency of these concentrations against fungal pathogens, noting that the ethanolic extract showed slightly higher effectiveness than petroleum ether extracts [20]. Although there have been no reported antifungal activities from flower extracts of *A. lebbeck* against keratinophilic fungi, the current study provides desirable data that can be used in formulating plant-based therapeutic products against the causative agents of these diseases.

The ethanolic extract was taken for analysis as it showed the highest inhibition rate against keratinophilic fungi. The results of the analysis of the crude alcoholic extract of *Albizia lebbeck* using GC–MS (gas chromatography-mass spectrometry) revealed the presence of several active compounds in the ethanolic extract, and upon examination, it was determined to consist of the following main composition as shown in Table (2).

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

able 2: The analysis of the crude alcoholic extract of *Albizia lebbeck* using GC–MS

	Compounds	Percentage
1.	n-hexadecanoicacid	(24.24%)
2.	oleic acid	(19.30%)
3.	9,12-octadecadienoic acid (Z,Z)	(10.04%)
4.	1,13-tetradecadiene	(6.59%)
5.	1-tridecyn-4-ol	(6.34%)
6.	methyl 2L, 9d-dimethyl teracosanoate	(5.08%)
7.	dodecanol	(2.94%)
8.	tridecene	(2.81%)
9.	bis-(3, 5, 5-trimethylhexyl) ether	(2.49%)
10.	3,3-difluro-1 tetradecen-4-ol	(2.20%)
11.	1 ethyldibenzothiophene	(2.07%)
12.	alpha.-Amyrin, trimethylsilyl etherD-Norandrostan-16-ol, acetate,	(15.8%)
	P-value	0.0001 **

**** (P≤0.01).**

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

N-Hexadecanoic acid is considered an antifungal against many types of fungi and is found in a certain group of plants, including the plant used in the study. Similarly, Oleic acid has the ability to inhibit fungal growth in addition to the virulence factors it possesses, which is consistent with [21, 22]. Regarding the experimental animals, the phenotypic results for the five groups taken from male mice, with each group containing 6 mice isolated in cages under the same environmental conditions, showed that the positive control mice, which were placed in a medium without pathogenic spores, did not suffer from any fungal diseases and were in good health. In contrast, the phenotypic results for the other four groups, which were exposed to keratinophilic fungal spores for one week at equal concentrations, revealed that all the mice developed cutaneous lesions ranging from mild to moderate after two weeks. After two weeks and after all the experimental mice were infected, the animals were treated with a 4 ml/L ethanolic extract of *Albizia lebbeck* plant, which showed inhibitory effectiveness against keratinophilic fungi.

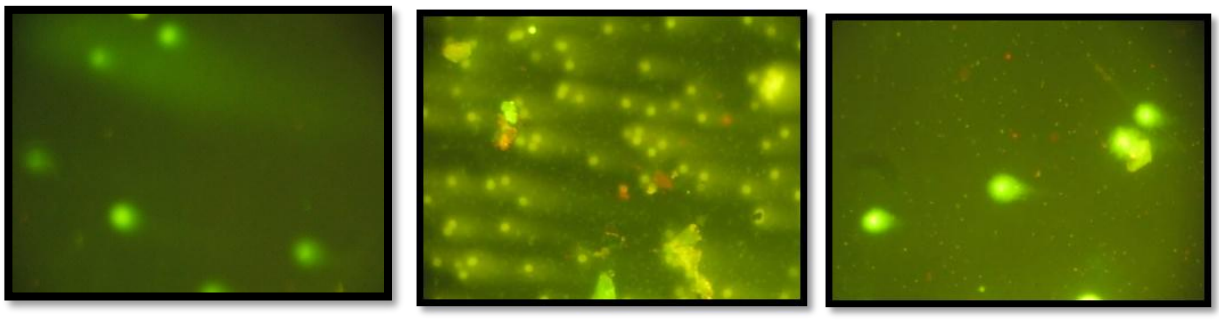
Later, blood cells (lymphocytes) from 30 mice were examined to conduct the comet assay three days after receiving treatment for the experimental animals. Documentation of single-cell generation was performed to detect the genetically variant forms of DNA that are affected if the treatment contains toxicity to the body. The different types of DNA typically consist of double-strand breaks, non-homologous DNA repair, unusual DNA, alkaline sites, and DNA cross-links. The analysis also allows the use of many sensitive reagents for any defect or anomaly that appears in the DNA taken from the sample blood cells (lymphocytes), and the test enables the quantification of DNA by measuring the amount of DNA or genetic contents and the resulting tail. The results in Table 3 show the control group that was not exposed to fungal infection, where there was no significant fluorescence of the tail, and the tail was not long. However, the percentage of modified and damaged DNA was evident in the group of mice that did not receive treatment, with noticeable fluorescence and a long tail indicating damaged and fragmented DNA. The third group, which received treatment after infection, showed a low

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

percentage of DNA damage and fragmentation, and the fluorescence was minimal, almost similar to the control group samples. This indicates the efficacy of the treatment from the plant extract and the recovery of the experimental mice without causing toxicity or harm to the test animal

Table 3: Measurement occurrence of DNA damages in Blood animals experiments and control using comet assay

	NO.	%	Low	%	Medium	%	High	%	Sum	Sum %
Control	72.00	44.40	69.00	42.13	11.66	7.10	10.33	6.36	163.00	100
Keratinophilic fungi	41.33	26.31	41.33	25.94	37.66	23.73	37.00	24.01	157.33	100
Treatment with *Albizia lebbeck* extract	74.67	49.20	41.33	27.39	19.66	13.04	15.66	10.35	151.33	100
P-value	0.0066 **	0.0002 **	0.0242 *	0.0009 **	0.0061 **	0.0001 **	0.0001 **	0.0001 **	0.0001 **	1.00 NS
*** (P≤0.05), ** (P≤0.01).**										



a- Control group b- Keratinophilic fungi group c-Treatment with *Albizia lebbeck*

Figure 2. The comet assay for (a: control cells, b: Keratinophilic fungi group, c: Treatment with *Albizia lebbeck*).

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

Among the features of the comet assay is the measurement of DNA damage in cells, as well as providing accurate data about eukaryotic cells [12, 23]. The resulting damage is measured using antioxidants and specific enzymes, and measurements are typically done using specialized incubators to obtain precise results [24, 25], especially in sites that the DNA of the enzyme is sensitive to the results of degradation appear as breaks in the DNA, and the more breaks, the higher the frequencies of the DNA as a result of its degradation. The image is analyzed using a special program on the computer. The results appear as shining tails; for example, in damaged samples, the shining tails are long and dense, and the opposite is true for normal DNA, this studies aligns with [26, 9]. The comet assay conducted on the blood of a group of mice used in the experiment shows that the analysis results before treatment indicated a high amount of comets in image 1B, which reflects the damage to white blood cells that were affected and DNA fragmentation due to skin fungal infection, unlike image 1C, which shows that the amount of comets significantly decreased after treatment with the alcoholic extract of the plant *Albizia lebbeck* L., indicating the repair carried out by the extract [23]

References

1- Al-Qaysi, A.S., Z. R. Al-Halbosi, and M.M.F. 2016. Antagonistic activity of Rhizospheric bacteria and Arbuscular Mycorrhiza isolated from Iraqi soil against fungal pathogen Macrophominaphaseolina. Journal of Pharmaceutical, Biological and Chemical Sciences, 7: 1154.

2- Al-Hayanni H. S. A., and H.M. El-Shora. 2021. Various Extracts of Some Medicinal Plants as Inhibitors for Beta-lactamase Activity. Baghdad Science Journal, 18 (1): 47-53.http://dx.doi.org/10.21123/bsj.2021.18.1.0047.

3- Naji, N. M., Abu Serag, N. A. Anmar M. K. Al-Maamori, A. M. K. and Naji, O. M. 2023. A Review on pharmacological and chemistry activity of Albizia Lebbeck (L.) benth. European Scholar Journal (ESJ), 4 (3): 92-96. 2023.

4- Al-Saeed, F.A., N. Saima, M.H. Saeed, R. Hussain, S. Iqbal, A. M.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

M. Chatha, Ab. Ghaffar, and R. Akram. 2023. Oxidative stress, antioxidant enzymes, genotoxicity and histopathological profile in oreochromis niloticus exposed to lufenuron. Pakistan Veterinary Journal, 43(1):160–166. http://doi: 10.29261/pakvetj/2023.012.

5- Alwash, M. J. B.; S. A. Shlahi, S.F. Hamad. 2018. Impact of different ploidy levels change on dianthalexin content in *Dianthus caryophyllus* L. Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 20 (2): 501-505.

6- Ogórek, R.; Suchodolski, J.; Piecuch, A.; Przywara, K. and Viš ˇ novská, Z. 2022. Keratinophilic and Keratinolytic Fungi in Cave Ecosystems: A Culture-Based Study of Brestovská Cave and Demänovská L'adová and Slobody Caves (Slovakia). Applied science, 12: 1-17.

7- Banothu, V., C. Neelagiri, U., Adepally, J. Lingam, and K. Bommareddy. 2017. Phytochemical screening and evaluation of in vitro antioxidant and antimicrobial activities of the indigenous medicinal plant albizia odoratissima. Pharmaceutical Biology, 55(1): 1155-1161. Http:// doi: 10.1080/13880209.2017.1291694.

8- Usman, M.; Khan, W. R.; Yousaf, N.; Akram, S.; Murtaza, G.; Kudus, K. A.; Allah Ditta, A.; Rosli, Z.; Rajpar, M. N. and Nazre, M. 2022. Exploring the Phytochemicals and Anti-Cancer Potential of the Members of Fabaceae Family: A Comprehensive Review. Molecules, 27: 1-21. https://doi.org/10.3390/molecules.

9- Balkrishna, A.; Chauhan, S. M.; Anurag Dabas, A. and Arya, V. 2022. A Comprehensive Insight into the Phytochemical, Pharmacological Potential, and Traditional Medicinal Uses of *Albizia lebbeck* (L.) Benth. Hindawi, 1-19. https://doi.org/10.1155/2022/5359669.

10- Habeeb, H.M., G.S. Salih, I.A. Hassan, S.F. Hamad, W.H. Abd Al-Kubaisy, and S. AL-D. A. SH. Al-Qaysi. 2024. In Vitro Investigation of Phytochemical, Antioxidant and Antimicrobial Activities of Harpagophytum procumbens seeds

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

extracts. Plant Protection, 08 (03): 457-467. http://DOI:10.33804/pp.008.03.5277.

11- Ibraheem, R. M.; A. A. Mhaweshandk. and W. Abood. 2018. Estimation of the whole flavonoid, antioxidant, anti-bacterial challenge concerning viola odorata (banafsha) methanolic extract. The Iraqi Journal of Agricultural Science, 49(4):655-662.

12- Mohammad, T. H.; Taha, Z. R.; Hamad, S. F.; Salim, D. H. and Khajeek, T. R. 2025. A Qualitative Study of Fungi Adhered to some Aquatic Plants in Baghdad, Al-jadriya. Iraqi Journal of Science, 2025, Vol. 66, No. 11, pp: 4855-4869. DOI: 10.24996/ijs.2025.66.11.14.

13- Lamyaa, A. F. H. M. Teeba, A.M. Hussain. and Z. H. Helal. 2017. Effect of Some Isolation Fungi from Iraq / Baghdad Hospitals Apparatus on Physiology of Liver, Kidney and Testis in Albino Mice. International Journal of Science and Research, 6 (9): 300-305.

14- Matloob, A. A. H., A.Y. Abid and K.Z. Khadhair. 2017. Efficiency of arbuscular mycorrhizal fungi and some species of plant growth promoting Rhizobacteria to control fungi Fusarium chlamydosporum causing agent of decline date palm off shoots. Iraqi Journal of Agricultural Sciences. 48(2): 507-519. https://doi.org/10.36103/ijas.v48i2.418.

15- Hansen, S. H., A. J. Pawlowicz , L. Kronberg , B. G. Kristine , O. Ann-Karin, G. Brunborg . 2018. Using the comet assay and lysis conditions to characterize DNA lesions from theacrylamide metabolite glycidamide. Mutagenesis 33(1): 31–39. http:// doi: 10.1093/mutage/gex036.

16- Muruzabal, D., A. Collins, and A. Azqueta. 2021. The enzyme-modified comet assay: past, present and future. Food Chemical Toxicology, 147, 111865. http:// doi: 10.1016/j.fct.2020.111865.

17- **SPSS (2019).** Statistical Packages of Social Sciences- SPSS/ IBM Statistics 26 step by step. 16th Edition.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

https://doi.org/10.4324/9780429056765.

18- **SPSS (2019).** Statistical Packages of Social Sciences- SPSS/ IBM Statistics 26 step by step. 16th Edition. https://doi.org/10.4324/9780429056765.

19- Shuping , D. S. S. and J. N. Eloff. 2017. The use of plants to protect plants and food against fungal pathogens: A review. African Journal of Traditional, Complementary and Alternative Medicines, 14(4): 120-127. http:// DOI: 10.21010/ajtcam.v14i4.14.

20- Teeba H. Mohammad. 2023. A Comet Assay for Candida glabrata Infection and Treatment by Penicillium chrysogenum – Derived AgNPs. Iraqi Journal of Agricultural Sciences, 54(4):950- 956. http:// doi.org/10.36103/ijas.v54i4.1784.

21- Umar H, D. Kavaz, and N. Rizaner. 2019. Biosynthesis of zinc oxide nanoparticles using albizia lebbeck stem bark, and evaluation of its antimicrobial, antioxidant, and cytotoxic activities on human breast cancer cell lines. International Journal of Nanomedicine, 14:87-100. http:// doi: 10.2147/IJN.S186888.

22- Yousif, A. A. and W. A. Hassan. 2023. HPLC analysis and antifungal activity of some plant extracts against decay apple fruits. Iraqi Journal of Agricultural Sciences. 54(1): 291-302. https://doi.org/10.36103/ijas.v54i1.1702.

23- Shehab, Z. H., T. H. Mohammad, and A. A. Tawfiq. 2024. Growth inhibition of Pseudomonas aeruginosa using products of some probiotic microorganisms and secondary metabolites of Commiphora myrrhaextracts estimated by GC-MS technique. Baghdad Science Journal, 21(11):3463-3475. DOI: 10.21123/bsj.2024.9704.

24- Devi, P.S.V.; Prasad, Y.G. Nomuraea Rileyi—A potential mycoinsecticide. In Biocontrol Potential and Its Exploitation in Sustainable Agriculture: Volume 2: Insect Pests; Upadhyay, R.K., Mukerji, K.G., Chamola, B.P., Eds.; Springer: Boston, MA, USA, 2001; pp. 23–38.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

25- Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., and Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical biology & drug design, 80(3), 434-439.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2, Issue 2
Page 78 - 91

Table 1: Anti-fungal activity of flowers extracts of *Albizia lebbeck* against Keratinophilic fungi on Müller-Hinton Agar.

Zone of inhibition(mm) on Müller-Hinton Agar (MHA) mg/L													P-value
Keratinophilic fungi	Etroleum ether				Ehloroform				Ethanol				
	0.5	1	2	4	0.5	1	2	4	0.5	1	2	4	-
Control	0	0	0	0	0	0	0	0	0	0	0	0	0 NS
Trichophyton Schoenleinii	4	6	9	11	2	4	7	9	7	9	11	14	0.006 **
Microsporum canis	3	5	8	10	5	7	10	12	7	10	12	15	0.002 **
Aspergillus flavus	3	6	8	10	3	5	8	10	6	8	11	13	0.006 **
P-value	0.037 *	0.008 **	0.002 **	0.002 **	0.035 *	0.009 *	0.004 **	0.001 **	0.008 **	0.001 **	0.001 **	0.001 **	---
*** (P≤0.05), ** (P≤0.01).**													