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LC-MS and FTIR-Based Characterization of Flavonoid-Rich Extract of *Hyoscyamus albus* L. Plant and its Multifunctional Bioactivities

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The current study aimed to determine the phytochemical composition of the flavonoid-rich extract of *Hyoscyamus albus* L. (FEHA), collected from the Khenchela region, and to investigate its multiple bioactivities. Phytochemical profiling was explored using LC-MS and FTIR. The antioxidant activity was assessed via five assays. Antiarthritic activity was evaluated using the albumin denaturation method. The antidiabetic effect was measured through the glucose uptake assay in yeast cells. Antibacterial activity was tested using the disk diffusion method against five bacterial strains and anti-inflammatory potential was evaluated via inhibition of protein denaturation using the BSA technique. LC-MS analysis identified 15 phenolic compounds, with Cirsiliol being the most abundant and FTIR spectra revealed the presence of phenols, alkanes, alkenes, alkynes, aliphatics, carboxylic acids, aromatics, and amides. Among the antioxidant tests, the reducing power method showed the highest activity. Significant antiarthritic activity was recorded, and promising antidiabetic effects were observed. The extract also exhibited notable anti-inflammatory activity and, at higher concentrations, zone of inhibition was also observed against *E. coli*. These findings suggest that *H. albus*. could be a valuable natural source of pharmacologically active compounds with potential therapeutic applications.

1. Introduction

White henbane, also known as *Hyoscyamus albus* L. (*H. albus* L.), a member of the Solanaceae family, is a perennial herb that attained a height around 40 to 50 cm and has lobed-margined leaves with a minor fleshy touch. This traditional medicinal plant can be found close to walls, and in extremely isolated regions and it is widespread in the Mediterranean basin.[1,2]

H. albus is renowned for its diverse pharmacological effects, which include its antioxidant, anticancer, antidepressive, cardioprotective, and other properties. These effects are attributed to its rich content of bioactive compounds.[3,4]

Among these compounds, secondary metabolites play a significant role. These are natural products synthesized by plants, known for their broad range of biological and pharmacological activities.[5–7] Many studies have focused on the isolation and characterization of these phytochemicals.[8,9] Secondary metabolites are crucial for plant defense and significantly contribute to the therapeutic efficacy of medicinal plants. In *H. albus*, they are primarily synthesized in response to biotic and abiotic stressors, offering valuable insights into the biochemical pathways involved in their accumulation.

Understanding these pathways enhances our knowledge of the plant's ecological role and informs agricultural practices aimed at optimizing the yield of bioactive compounds.

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Recent studies suggest that conditions such as soil composition, light availability, and moisture levels significantly influence the production of secondary metabolites in *H. albus*.[5,6,10]

Flavonoids, one of the most prevalent classes of secondary metabolites, play a pivotal role in the medicinal properties of *H. albus*. These compounds have drawn attention due to their multifaceted health benefits, including anti-inflammatory, anticancer, and antiviral activities.[9,11,12] Their structural diversity and interaction with various biological targets underscore their pharmacological potential. Studies show that the extraction and purification of flavonoids from *H. albus* can yield potent bioactive agents.[13]

The application of advanced analytical techniques such as LC-MS and FTIR represents a major advancement in phytochemical research.[14] These methods enable comprehensive profiling of complex plant extracts and can effectively identify key bioactive compounds.[15] For instance, LC-MS has successfully identified multiple flavonoids and phenolic acids in *H. albus*, known for their antioxidant activity and their ability to mitigate oxidative stress.[16] FTIR analysis further helps in determining functional groups in these compounds, thus enhancing our understanding of their therapeutic potential.[17]

Given the pharmacological promise of *H. albus*, further investigation is warranted. Integrating traditional botanical knowledge with modern scientific tools could pave the way for novel drug development and provide empirical validation of its traditional uses.

In this context, the present study focuses on the Khenchela region, aiming to determine the phytochemical composition of FEHA using LC-MS and FTIR techniques, and evaluate its biological activities, thereby offering scientific proof for the traditional medicine uses.

2. Materials and Methods

2.1. Plant Material

The *H. albus* aerial parts (Figure 1) were harvested in May 2018 at Djellal region located approximately 65 km from the wilaya of Khenchela eastern Algeria (Latitude: 34.9167, Longitude: 6.9, 34° 55′ 0″ North, 6° 54′ 0″ East). The taxonomic identification of the plant was confirmed by a specialist Dr. Azzeddine ZERAIB professor at the Department of Agronomy, Khenchela University. To comply with strict scientific procedures, the Laboratory of Biotechnology, Water, Environment, and Health assigned a unique voucher code (No.454/40) to the plant specimen.

The plant was dried at room temperature for 15 days until it completely dehydrated, and then ground it into a fine powder. The powder was then stored in a dark and dry place to prevent the damaging effects of humidity.

2.2. Preparation of Flavonoid-rich Extract of *H. albus*

The extraction of flavonoids from the aerial parts of *H. albus* was performed using maceration method slightly modified adapted from.[18]

A 10 g of a powdered areal part of *H. albus* plant was extracted in 100 mL of 70% aqueous ethanol (ethanol: distilled water, 70:30 v/v) for three days with the renewal of the solvent every 24 h. Next, the extract was filtered and concentrated using a rotary evaporator, drying them at 40 °C. The residue was dissolved in boiling distilled water (50 mL) for 12 h. After cooling, the mixture was filtered through Whatman No. 1 filter paper to remove solid residues. The resulting aqueous extract was then subjected to liquid–liquid extraction with *n*-butanol (3 × 50 mL) to isolate the flavonoid-rich fraction, exploiting the differential solubility of phenolic compounds in organic solvents. The n-butanol layers were combined and evaporated to dryness under reduced pressure at 40 °C using a rotary evaporator. The dried FEHA was stored at 4 °C until further analysis.

2.3. Quantitative and Qualitative Characterization of FEHA

2.3.1. Liquid Chromatography Mass Spectrometry (LC-MS) Analysis

The FEHA LC-MS analysis was conducted using the Shimadzu UFLC XR system (Kyoto, Japan), which included an autosampler (SIL-20AXR), column oven (CTO-20 AC), binary pump (LC-20ADXR), detector system (quadripole 2020), and Inert sill column (ODS-4 C18 3 µm). The column temperature was set at 40 °C and the injection volume at 20 l (0.5 mL/min). Two mobile phases were employed: A (95% H_2O, 5% Methanol, and 0.2% Acetic acid) and B (50% ACN, 50% H_2O, and 0.2% acetic acid). The elution gradient was outlined as follows: 10%–20% B (0.01–14 min); 20%–55% B (14–27 min); 55%–100% B (27–37 min); 100% B (37–45 min); and 10% B (45–50 min), with a flow rate of 1.50 L/min for the nebulizing gas, 275 °C for the dissolving line temperature, 15.00 L/min for the drying gas, and 450 °C for the heat block temperature. LC-ESI (-) MS mass spectra (M-H) were acquired using Lab Solutions software and identified compounds by comparing retention times to those of reference standards.

2.3.2. Fourier Transform Infrared (FTIR) spectroscopy

FEHA was subjected to FTIR analysis using a Perkin–Elmer spectrometer (Perkin–Elmer, USA) as described by Sahoo and Marakanam.[19] An isostatic press of 150 MPa was used to turn 100 mg of KBr and 10 mg of the extract into a thin pellet that was 200 to 300 µm in size. This pellet was then put into the FTIR spectrometer. The infrared spectra were obtained in the wavelength range 400–4000 cm^{-1} with a resolution of 4 cm^{-1} at room temperature. The different functional groups present in the tested extract were identified from the spectral data by comparing it with a reference.

2.4. Biological Activities

2.4.1. Antioxidant Activity

DPPH radical scavenging assay: DPPH radical-scavenging activity of FEHA was determined using a slightly modified version of

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Figure 1. Aerial part of *H. albus* L.

Lai's method.[20] To begin, 1 mL of 0.1 mmol/L of DPPH solution freshly prepared was mixed with 3 mL of various concentrations of FEHA. The mixture is vigorously vortexed and subsequently incubated in the dark at room temperature for 30 min. Also, 3 mL of ethanol was used as a control. The absorbance was measured at 517 nm and the assays were conducted in triplicate. The following formula was used to determine the DPPH radical–scavenging activity:

$$\text{Radical scavenging activity (\%)} = (\text{AC} - \text{AS}/\text{AC}) \times 100$$

where (AC) is the absorbance of the control (which contains all reagents excluding the test sample) and AS is the absorbance of the FEHA.

Analyses were performed in triplicate and the results were expressed as average values with standard deviation (S.D.). Ascorbic acid was used as the positive control. This data was used to draw the curve and determine the inhibitory concentration (IC_{50}) value.

Ferric-reducing power assay: The reducing power of FEHA was estimated using Oyaizu (1986) procedure.[21] 2.5 mL of different concentrations of FEHA was mixed with 2.5 mL of sodium phosphate buffer (0.2 mol/l, pH 6.6) in triplicate, then 2.5 mL of potassium ferricyanide (1%) was added. The mixture was incubated at 50 °C for 20 min, after which 2.5 mL of 10% trichloroacetic acid was added after the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5) was mixed with distilled water (5 mL) and 0.1% ferric chloride (1 mL). After 30 min of incubation, the absorbance values were readied at 700 nm (higher absorbance indicates higher reducing power). Each experiment was conducted at least three times for both the standard and the sample. The reducing power activity was calculated using the following formula:

$$\text{Reducing power (\%)} = (\text{Abs blank} - \text{Abs E}/\text{Abs blank}) \times 100$$

where Abs blank is the absorbance of the negative control (containing all reagents except the test sample) and Abs E is the absorbance of the FEHA.

The results were expressed as IC_{50}. Ascorbic acid was tested in the same conditions at different concentrations to calculate Ferric-reducing power values for tested extract.

Phosphomolybdenum assay: The total antioxidant capacity (TAC) of FEHA was assessed using the phosphomolybdenum method slightly modified as reported by Zengin et al.[22] The FEHA (0.3 mL) was blended with 3 mL of reagent mixture (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The absorbance was spectrophotometrically determined at 695 nm after 90 min of incubation at 95 °C and cooling to room temperature. The FEHA is replaced with ethanol as negative control and ascorbic acid is used as a positive control. The following formula was used to calculate the total antioxidant activity:

$$\text{The total antioxidant activity (\%)} = (\text{Abs blank} - \text{Abs E}/\text{Abs blank}) \times 100$$

where Abs blank is the absorbance of the negative control (containing all reagents except the test sample) and Abs E is the absorbance of the FEHA. The total antioxidant activity is expressed as IC_{50}.

Hydrogen peroxide scavenging assay: The Ruch method[23] was used to subject the FEHA to a hydrogen peroxide scavenging assay. A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4). The FEHA at (50–250 mg/mL) was added to hydrogen peroxide solution (0.6 mL) and allowed to stand at room temperature for 15 min. At 230 nm, the absorbance was measured in comparison to reagent blank containing only phosphate buffer. A distinct blank sample was used for background subtraction for each concentration. each sample's concentration was examined in triplicate. Ascorbic acid

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was chosen as the positive control. the following formula was used to calculate the percentage inhibitory activity:

$$\text{Inhibitory activity\%} = \left(1 - \text{absorbance of sample} / \text{absorbance of control}\right) \times 100$$

The inhibitory concentration (IC_{50}) value was calculated.

β-carotene-linoleic acid bleaching assays: The FEHA was subjected to β-carotene-linoleic acid bleaching assay as reported by[23] method slightly modified.

A 10 mL of chloroform was used to dissolve10 mg of β-carotene, and then the excess solvent was removed with a rotary vacuum. An orange emulsion was developed by mixing 1 mL of the previous solution with 200 µL of linoleic acid, 2 mL of Tween 20, and 50 mL of distilled water. Each test tube contained 200 µL of the relevant FEHA at various concentrations and 4.8 mL of the emulsion.

Following an immediate ($t = 0$) spectrophotometer measurement of the mixtures' absorbance at 470 nm, they were left to incubate for 50 min in a water bath. Each 30–120 min, the absorbance was recorded.

Using the following formula, the antioxidant activity coefficient (AAC) was calculated in relation to the bleaching of β -carotene:

$$\text{AAC} = 1000 * (\text{Abs Sample (120 min)} - \text{Abs Control (120 min)}) / (\text{Abs Control (0 min)} - \text{Abs Control (120 min)})$$

Then, the IC_{50} value was calculated.

2.4.2. Antiarthritic Assay

The protein denaturation method was used to evaluate the antiarthritic effects of FEHA as described by Mizushima and Kobayashi[24] with few modifications.

A 0.2 mL of egg albumin, 2.8 mL of phosphate buffer saline (pH 6.4), and 2 mL of FEHA at various concentrations were mixed and incubated for 15 min at 37 °C. After that, the mixture was heated for 5 min at 70 °C then let it cool.

Distilled water was used as control and the vehicle as blank. The absorbance was recorded at 660 nm.

The % of inhibition of protein denaturation was measured by the follow formula:

$$\text{Inhibition\%} = \left[(\text{Abs control} - \text{Abs sample}) / \text{Abs control}\right] \times 100$$

where: Abs control: is the absorbance of control solution, Abs sample: Absorbance of the test sample.

The IC_{50} value was calculated.

2.4.3. Antidiabetic Activity (Glucose uptake in yeast cells)

Glucose uptake test by yeast cells for FEHA was assayed according to Cirillo et al.[25] procedure with minor modifications. The yeast suspension (*Saccharomyces cerevisiae*) was repeatedly centrifuged at 3000 g for 5 min until a clear supernatant fluid was obtained. 10% (v/v) of the cleared yeast suspension was prepared in distilled water.

1 mL of FEHA (0.25–5 mg/mL) was added to 1 mL of glucose solution (5, 10, and 25 mM) and incubated at 37 °C for 10 min. To start the reaction, 100 µL of yeast suspension was added, and then mixed all. All tubes were centrifuged (2500 g for 5 min) after an hour of incubation at 37 °C. The supernatant was used to measure the absorbance at 540 nm, estimating the quantity of glucose in each tube. Metronidazole drug was considered as standard. All tests were achieved in triplicates. The increase in glucose uptake was calculated using the next formula:

$$\text{Increase in glucose uptake} = \left[(\text{Abs control} - \text{Abs sample}) / \text{Abs control}\right] \times 100$$

where: Abs sample: is the absorbance of test sample, Abs control: is the absorbance of control reaction (containing all reagents except the test sample).

The IC50 value was calculated.

2.4.4. Anti-inflammatory Activity

The inhibition of protein denaturation of FEHA was the method used to estimate the anti-inflammatory activity as given by Reshma Arun and Brindha[26] with minor modification.

Test solution consists of 0.45 mL of BSA (5 %) and 0.05 mL of FEHA (250 µg/mL). The pH of the above mixture was adjusted to 6.3 by hydrochloric acid (1 N) then incubated at 37 °C for 20 min. Afterward, the mixture was placed in a water bath for 3min at 57 °C to cause the protein denaturation, following by cooling at room temperature. 2.5 mL of phosphate buffer saline was then added. The absorbance values of test solution were read at 416 nm.

The control solution was constituted of distilled water and BSA. The product control solution comprised distilled water and test solution. The standard solution contained diclofenac sodium with BSA.

The inhibition percentage was calculated asfollows :

$$(\text{Ac} - (\text{As} - \text{Ap}) / \text{Ac}) \times 100$$

where: As is the absorbance of the sample, Ap is the absorbance of the product control and Ac is the absorbance of the positive control. All measures were repeated three times. The IC_{50} value was calculated.

2.4.5. Antibacterial Activity

The FEHA was tested for antibacterial activity by disk diffusion method. Five germs were tested: *Staphylococcus aureus* (*S.aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae*(*K. pneumoniae*), and *Enterobacter*.

Briefly, the discs (sterile filter paper of 6 mm diameter) were impregnated with 10 µL of the test sample and placed on the

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Table 1. Phenolic acids identified by LC-MS in the FEHA.

ID	Name	Retention Time	*m/z*	Concent-ration	Unit
1	Quinic acid	2.239	191.00	15.562	ppm
2	Gallic acid	2.233	169.00	0.249	ppm
3	Protocatechuic acid	6.963	153.00	5.048	ppm
4	Caffeic acid	15.856	179.00	4.037	ppm
5	Syringic acid	17.954	197.00	8.764	ppm
6	P-Coumaric acid	22.758	163.00	4.256	ppm
7	trans-Ferulic acid	25.452	193.00	0.742	ppm
8	Hyperoside (quercetin-3-O-galactoside)	28.121	463.00	1.927	ppm
9	Naringin	29.651	579.00	3.200	ppm
10	Quercitrin (quercetin-3-O-rhamnoside)	30.369	447.00	1.319	ppm
11	Apigenin-7-O-glucoside	30.506	431.00	0.917	ppm
12	Quercetin	34.871	301.00	0.551	ppm
13	Kaempferol	38.011	285.00	0.191	ppm
14	Luteolin	37.979	285.00	0.154	ppm
15	Cirsiliol	38.842	329.00	71.636	ppm

inoculated agar plates. The FEHA was prepared at 10 mg/mL by dimethyl Sulfoxide (DMSO). The petri plates were incubated for 24 h at 37 °C.

The antibacterial activity was determined by measuring the inhibition zone diameter (mm) that surrounds the discs. The experiment was carried out twice. Gentamycine and Ofloxacine antibiotics were the positive controls and DMSO the negative control.

2.5. Statistical Analysis

Data obtained were presented as (mean ± standard) deviation of three dependent determinations. Significant differences between the means of total flavonoids and LC-MS analysis results were determined by Student t-test, and *p*-values (<0.05) were regarded as significant. Results of antioxidant, anti-inflammatory, antidiabetic, and antiarthritic activities were expressed as "inhibitory concentration (IC_{50})" values which were calculated using Microsoft Excel 2010 from linear regression analysis of percent activity as a function of concentration. Obtained data were subjected to statistical analysis of variance (one factor) using SAS (9.1.3) package at $p < 0.05$ significant levels.

3. Results and Discussion

3.1. LC-MS Analysis

The compounds identified by LC-MS in FEHA are listed in Table 1. The LC-MS characterization of phenolic acids that were found in the FEHA has identified 15 phenolic acids, which are Quinic acid, Gallic acid, Hyperoside (Quercetin-3-O-galactoside), Caffeic acid, P-Coumaric acid, trans-Ferulic acid, Syringic acid, Protocatechuic acid, Naringin, Apigenin-7-O-glucoside, Quercitrin (quercetin-3-O-rhamnoside), Quercetin, Cirsiliol, Luteolin et Kaempferol ; in which the principal compound is Cirsiliol with a concentration of 71.636 ppm, then the Quinic acid with a concentration of 15.562 ppm.

Many research papers have reported the biological potential of Cirsiliol, which was known in traditional medicine as antioxidant, antibacterial, anti-inflammatory, and antiproliferative.[27–29]

In addition, Quinic acid and Syringic acid were found to be the responsible for many biological activities as anti-inflammatory, antioxidant, antiproliferative, antibiofilm, antibacterial, antifungal, antihyperglycemic, hepatoprotective, antidiabetic, anticancer, antiviral, antinociceptive, and analgesic effects.[30–34]

The comparison of our findings with previous works will be so difficult due to rare data in literature about this kind of analysis for this plant.

Only one preceding study[4] which reported the phenolic profile of *H. albus* extract using LC-MS method, and could identify as common compounds Quinic acid, Protocatechuic acid, Caffeic acid, P-Coumaric acid, trans-Ferulic acid, Quercetin-3-O-galactoside, Quercetin-3-O-rhamnoside, and Luteolin.

However, the HPLC analysis of *Hyoscyam muticus* (*H. muticus*) could identify, as common phenolic compounds, Gallic acid, trans-Ferulic acid, Luteolin, and Apigenin-7-O-glucoside,[35] trans-Ferulic acid and Quinic acid,[3] Gallic acid, P-Coumaric, Caffeic acid, and Quercitrin.[36]

On the other hand, Tlili et al. identified 11 phenolic compounds in *H. albus*'s acetonic extract.[4] Ayari-Guentri et al. found 19 phenolic compounds in the *H. muticus* methanolic leaf extract.[35] Also, Elsharkawy et al. found in *H. muticus* methanolic extract of aerial parts eight phenolic compounds.[3] Besides, Kebaili et al. registered high phenolic content in ethanolic extract compared to acetone and methanol in *Hyoscyamus reticulates (H. reticulalates)*.[37]

So, many factors may affect the phytochemicals composition of the extract; like the nature of solvents and extraction methods,[38,39] geography and weather of the area,[3,37] plant species and the part concerned by the study.[37,40]

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Table 2. FTIR interpretation of compounds of FEHA.

Peak Values (cm^{-1})	Vibration	Functional Group Assignment[a)]
3400	O—H stretching	Alcohols, phenols
3000	O—H stretching	Alkene
2925	C—H stretching	Aliphalics Alkane
2850	C—H stretching	Aliphalics Carboxylicacid
2100	C≡C	Alkyne
1725	C═O stretching	Amide I (peptide bond)
1675	C═O stretching	Amide I (peptide bond)
1648	C═O and C—N stretching	Amide I (peptide bond)
1550	C—N stretching N—H bending	Amide II
1500	C—N stretching N—H bending	Amide II
1475	CH2 bending	Aromatics
1450	C—N stretching N—H bending	Amide III Aromatics
1300–1375	O—H bend, alcoholic group	Phenolics and aliphatics
1200–1254	PO^{2}-stretching (asym.) C═O	Amide III Carboxylicacid
1154–1150	PO^{2}-stretching (asym.)	Amide III Carbohydrates
1075	C—O stretching	Aliphalic amines Carbohydrate
1025	C—O stretching	Carbohydrates Aliphalic amines
900–950	C—OH C—O—C C—C P═O	Polysaccharides Phosphodiesters Carbohydrates
800–875	C—O stretching	Saturatedprimaryalcohols carbohydrates
775–600	O—C—O bending	Halogene compounds(C—Cl, C—F, C—Br)

[a)] Assignment was executed according to.[17,19–41–46]

3.2. FTIR Analysis

A spectroscopic analysis was performed for the first time on FEHA. Comparing the FTIR spectrum with the reference chart revealed the presence of phenols, alkanes, alkenes, aliphalics, carboxylic acids, aromatics, and amides in the *n*-butanol extract (Table 2 and Figure 2). LC-MS has already confirmed the presence of phenolic compounds.

3.3. Evaluation of Antioxidant Activity

The one-factor analysis of variance (Table 3) has revealed that the difference between the antioxidant activity methods used (DPPH, TAC, H_2O_2, β-carotene, and RP) was very highly significant ($p \leq 0.001$).

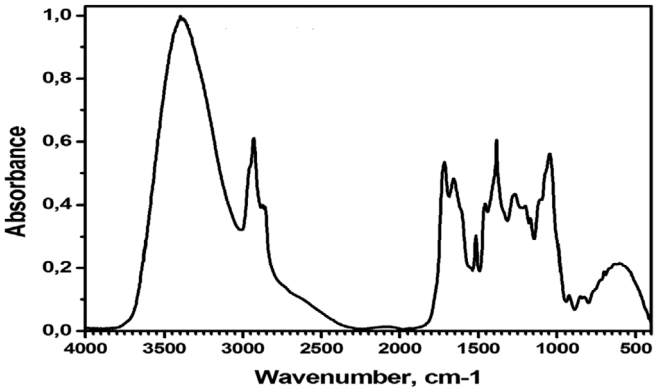


Figure 2. FTIR spectra of FEHA.

The average results of the antioxidant activity by the five methods are shown in Table 4.

The minor significant difference (LSD = 0.027) was disclosed 5 groups of antioxidant activity methods, where the RP method was the best one to evaluate the antioxidant activity of FEHA (IC_{50} = 0.017 ± 0.00013 mg/mL), then followed by DPPH, β-carotene, H_2O_2, and TAC methods with (IC_{50} = 0.22 ± 0.05 mg/mL), (IC_{50} = 4.13 ± 0.03 mg/mL), (IC_{50} = 7.64 ± 0.011 mg/mL), and (IC_{50} = 9.27 ± 0.008 mg/mL), respectively, compared to ascorbic acid as a standard with IC_{50} equal to 0.06 ± 0.005, 0.09 ± 0.05, 4.6 ± 0.1, 0.11 ± 0.01, and 0.16 ± 0.03 mg/mL, respectively, for RP, DPPH, β-carotene, H_2O_2, and TAC. These results indicate that the FEHA exhibits the most effective activity by RP method in comparison to the standard used by the same method.

The *H. albus* L. extract showed an important antioxidant activity by all antioxidant tests with slight differences. This remarkable capacity was interpreted by their chemical composition as shown in this work, principally Cirsiliol, Quinic acid, and Syringic acid, which have been proven they antioxidant potential in much previous research.[29,32–34]

In addition, many reports have been proved the high correlation of phenolic acid, their specific positions of hydroxyl groups, number, and nature of the substitutions with the antioxidant potential.[47–50]

Concerning the differences that have been found, it may be attributed to the variation in mechanisms of reaction of each assay.[51,52] However, Barchan et al. have reported that the relationship between phenolic acids and antioxidant activity is not constantly present or depends on the evaluation method of the antioxidant activity.[53]

3.4. Evaluation of Antiarthritic Activity (Protein Denaturation Method)

The one-factor analysis of variance (Table 5) has showed a very highly significant difference between the method used to evaluate the antiarthritic activity and the standard (Diclofinac) ($p \leq 0.001$).

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Table 3. Analysis of variance of the antioxidant activity of FEHA.

Source	DF	Sum of Squares	Mean Square	*F*-value	Pr > F
antioxidant activity	4	212.575	53.1439	225812	<0.0001
Error	10	0.00235	0.0023		
Corrected total	14	212.578			

*** : very highly significant at 5%.

Table 4. Evaluation of the antioxidant activity of FEHA.

	DPPH IC_{50} (mg/mL)	B-Carotene IC_{50} (mg/mL)	TAC IC_{50} (mg/mL)	RP IC_{50} (mg/mL)	H_2O_2 IC_{50} (mg/mL)
FEHA	0.22 ± 0.05 d	4.13 ± 0.03 c	9.27 ± 0.00 a	0.01 ± 0.00 e	7.64 ± 0.01b
Ascorbic acid	0.09 ± 0.05	4.60 ± 0.10	0.16 ± 0.03	0.06 ± 0.00	0.11 ± 0.01

TAC: Total antioxidant Capacity; **RP**: Reducing Power; **a, b, c, d**: Homogenous group

Table 5. Analysis of variance of the antiarthritic activity of FEHA.

Source	DF	Sum of Squares	Mean Square	*F*-value	Pr > F
Antioxidant method	1	52.6288	52.6288	24669.8	<0.0001
Error	4	0.0085	0.0021		
Corrected total	8	52.6373			

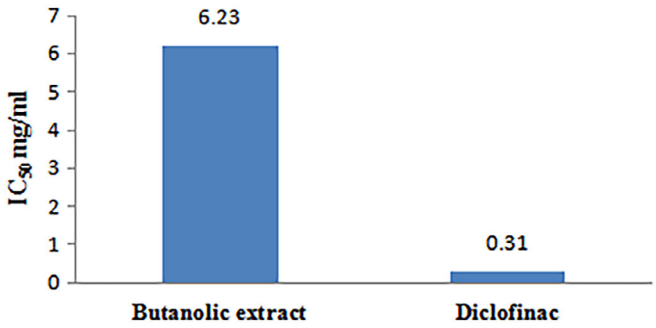


Figure 3. The antiarthritic activity of FEHA.

The results of antiarthritic activity of FEHA are shown in Figure 3. Considerable activity was recorded (IC_{50} = 6.23 ± 0.05 mg/mL) in comparison to Diclofinac as control (0.31 ± 0.04 mg/mL).

Regarding these findings, FEHA inhibits significantly the denaturation of protein ($P \leq 0.001$) in comparison with Diclofinac.

There are no researches existing previously in literature about *H. albus* antiarthritic activity, so it's not easy to make a comparison with our findings. However, these observations could be interpreted by the different phytoconstituents of the plant.[54] Also, the inhibition of the denaturation mechanism involving the alteration of various bonds; hydrogenic, hydrophobic, electrostatic, and disulfide could be the main reason of this antiarthritic effect.

The present findings were in concordance with those of Alamgeer and Hasan,[55] which found that the flavonoids fraction of *Berberis orthobotrys* gave the highest activity (92.60%) compared to the other fractions (aqueous-methanolic extract (30:70), ethyl acetate and aqueous).

Table 6. Analysis of variance of the antidiabetic activity of FEHA.

Source	DF	Sum of Squares	Mean Square	*F*-value	Pr > F
Antidiabetic	2	1.4478	0.723	24669.8	<0.0001
Error	6	0.0004	0.0000		
Corrected total	8	1.4482			

3.5. Evaluation of the Antidiabetic Activity

The one-factor analysis of variance (Table 6) showed that there was a very highly significant difference between the concentrations of FEHA ($p \leq 0.001$) regarding to the antidiabetic activity.

According to the outcomes observed in Figure 4, the FEHA has demonstrated a good antidiabetic activity with IC_{50} equal to 0.27 ± 0.014 mg/mL, 1.25 ± 0.014 mg/mL, and 0.68 ± 0.028 mg/mL, respectively, for 5, 10, and 25 mM, compared to Metronidazol (standard) which showed IC_{50} egal to 1.46 ± 0.09 mg/mL, 0.33 ± 0.012 mg/mL, and 0.21 ± 0.006 mg/mL for the same concentrations, respectively (Figure 4).

These current results are not capable of comparing with previous results due to the absence of data in the literature for our plant species.

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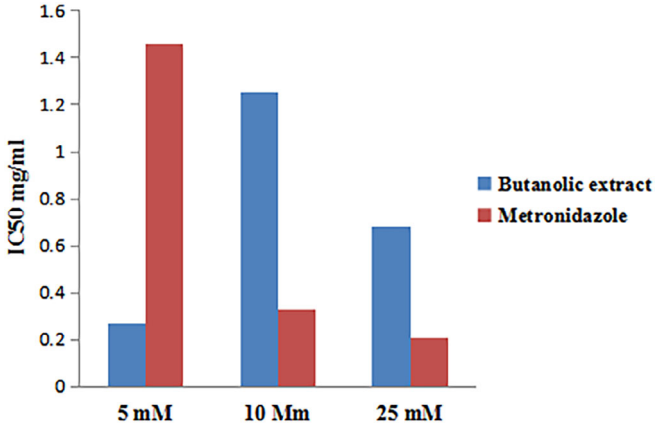


Figure 4. Antidiabetic activity of FEHA.

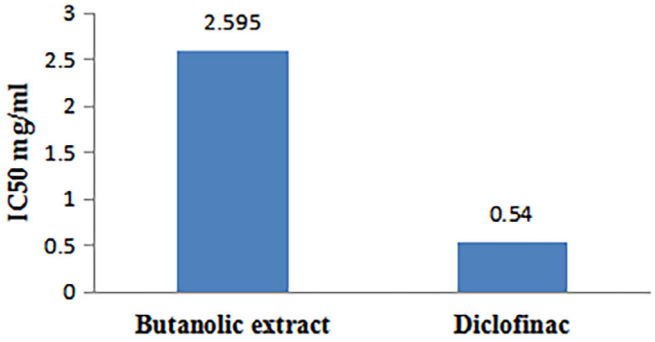


Figure 5. The anti-inflammatory activity of FEHA.

Table 7. Analysis of variance of the anti-inflammatory activity of FEHA.

Source	DF	Sum of Squares	Mean Square	*F*-value	Pr > F
Anti-inflammatory	1	6.4273	6.4273	9181.93	<0.0001
Error	4	0.0028	0.0007		
Corrected total	5	6.4301			

However, findings acquired in the present paper could be interpreted by the mixture of phytochemicals present in the extract, or a particular compound as phenolic compounds, flavonoids and others syringic acid and ferulic acid,[56] Luteolin,[57] Naringin,[34,58] and Kaempferol[34,59] were found to acquire antidiabetic properties (protective and therapeutic effects).

The effect of reducing blood glucose level by this phytochemical was exerted through diverse mechanisms like regulation of glucose and lipid metabolism, cells stimulation, insulin secretion, inhibition of gluconeogenic enzymes, and protection against reactive oxygen species (ROS).[60,61]

In the context of these results, *H. albus*. plant could be considered as new alternative for diabetes type 2 treatment because of their phytochemical's composition.

3.6. Evaluation of the Anti-inflammatory Activity

The one-factor analysis of variance (Table 7) showed that there was a very highly significant difference between FEHA and Diclofinac (standard used) ($p \leq 0.001$) regarding to the anti-inflammatory activity.

According to the found results shown in Figure 5, the FEHA registered an IC_{50} equal to 2.59 ± 0.03 mg/mL. On the other hand, Diclofenac sodium drug, which is used as standard, exhibited IC_{50} equal to 0.54 ± 0.01 mg/mL.

These results could be related to antioxidant property and the high content of phenolic compounds of the extract, such as polyphenols and flavonoids, known by its anti-inflammatory activity with several mechanisms actions, which was in compliant with those found by Gunathilake et al.[62] and Cherbal et al.[63]

Also, Djuichou et al. was found to have a high correlation between concentration of phytochemicals and percentage of protection.[54]

In addition, the chemical composition of *H. albus* extract detected by LC/MS, have been proven they anti-inflammatory activity in many previous researches, mainly Cirsiliol,[29,64,65] Quinic acid,[32,66] and Syringic acid,[32,67] which could explain this remarkable capacity.

3.7. Evaluation of Antibacterial Activity

The one-factor analysis of variance (Table 8) showed that there was a very highly significant difference in the diameter of the zone of inhibition between the bacterial strains and between the bacteria and the positive (Gentamycin and Ofloxacin) and standard negative (DMSO) controls ($p \leq 0.001$).

The mean diameters of the zone of inhibition of FEHA and the positive and negative controls against five bacterial strains (*S. aureus, K. pneumoniae, E. coli, Enterobacter*, and *P. aeruginosa*) are presented in Table 9.

Evaluation of antibacterial activity by the disc method showed that the zone of inhibition was changed depending on concentration of the extract and from one strain to another (Table 8).

According to the present observations, the FEHA at 5 mg/mL did not have antibacterial activity against the five bacterial strains tested. However, a large zone of inhibition was observed (12.33 ± 0.5 mm and 9 ± 1 mm) for *E. coli* strain at 100 and 10 mg/mL, respectively. Therefore, *E. coli* is considered as the most sensitive strain among those tested.

Literature on *H. albus* antibacterial proprieties are insufficient, so comparison with other species from the same family might be a good approach to confirm the agreement of our findings with preceding reports on different *Hyoscyamus* species.

No antibacterial activity of *H. albus* ethanolic extract against most of the tasted strains was demonstrated in the report of Elsharkawy et al.[3] However, the study of Kadi et al. was found an antibacterial activity with inhibition zone equal to 7.0 to 14.0 mm attributed to alkaloids fractions of the same plant.[68]

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Table 8. Mean squares of the variance analysis of the method effect on antibacterial activity.

Source	DF	*S. aureus*	*K. pneumoniae*	*E. coli*	*Enterobacter*	*P. aeruginosa*
Antibacterial activity	5	261.4610***	444.4805 ***	492.7222***	460.0888***	176.8488***
Error	17	0.3416	0.4877	0.7777	0.4672	0.2300

*** very highly significant at 5%.

Table 9. The mean diameters of the zone of inhibition.

FEHA	Concentrations (mg/mL)	Zone of inhibition (mm) (mean ± SD)				
		S. aureus	*K. pneumoniae*	*E. coli*	*Enterobacter*	*P. aeruginosa*
	5	–	–	–	–	–
	10	06.1 ± 0.1 d	07.0 ± 0.1 d	09.0 ± 1.0 d	07.0 ± 0.1 d	07.63 ± 0.1 c
	100	08.2 ± 0.2 c	08.2 ± 0.5 c	12.3 ± 0.5 c	08.2 ± 0.2 c	08.16 ± 0.1 c
Gentamycine		20.0 ± 1.0 b	21.5 ± 0.5 b	30.0 ± 1.0 a	31.5 ± 1.3 a	14.50 ± 0.5 b
Ofloxacine		21.0 ± 1.0 a	30.3 ± 1.5 a	26.3 ± 1.5 b	20.0 ± 1.0 b	19.16 ± 1.0 a
DMSO		–	–	–	–	–
LSD		1.039	1.2425	1.5689	1.216	0.8532

(-): no inhibition zone.

3.7.1. Antibacterial Activity Against Gram-Positive Bacteria

In a related study, methanolic extracts of *H. muticus* demonstrated average activity against Gram-positive bacteria, with inhibition zones recorded as 13.0 ± 1.0 mm (*S. aureus*), 11.75 ± 0.25 mm (*S. epidermidis*), and 11.5 ± 0.5 mm (*B. cereus*).[3] Notably, Dulger et al. reported strong antibacterial activity (25.0 mm inhibition zone) against *S. aureus* using a hydromethanolic extract of *H. niger* seeds.[69]

3.7.2. Antibacterial Activity Against Gram-Negative Bacteria

In contrast, *H. muticus* methanolic extracts showed relatively weak activity against Gram-negative bacteria, with inhibition zones ranging from 6.5 ± 0.5 mm (*K. pneumoniae*) to 12.0 ± 0.0 mm (*P. aeruginosa*).[3] The current study's observation of moderate activity against *E. coli* aligns with these earlier findings.

3.7.3. Contribution of Phenolic Compounds

The antibacterial potential of FEHA may be attributed to its phenolic and flavonoid content. Previous studies have shown that compounds such as quercetin, flavone, and naringenin possess significant antibacterial activity, particularly against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. epidermidis*, and *A. niger*.[70] Additionally, specific phenolic acids identified in the extract such as quinic acid, syringic acid, and Cirsiliol- have been reported to exhibit antimicrobial properties.[29,31,32,37] Gram-positive bacteria generally appear more susceptible to these compounds than Gram-negative strains, likely due to differences in cell wall composition.

These results may support the traditional use of *H. albus* for antimicrobial purposes, especially against Gram-negative bacteria such as *E. coli*, although further investigation and compound isolation are warranted to clarify the specific contributors to its bioactivity.

4. Conclusion

In our study, some biological activities of flavonoid-rich extract of *H. albus* L. plant belonging to Khenchela region (Algeria) were investigated. The results concluded that our extract contained many phytochemical compounds known by their high bioactivities, such as Cirsiliol, Quinic acid, and Syringic acid, confirming their presence by LC-MS and FTIR analysis. The RP method was the best one to evaluate the antioxidant activity of FEHA, and then followed by DPPH, b-carotene, H_2O_2, and TAC methods. Considerable antiarthritic activity was registered for the extract which was found to inhibit significantly the denaturation of protein. Also, for the antidiabetic activity, good activity at different concentrations. The studied extract exerted a significant increase of inhibition of protein denaturation at different concentrations. Concerning the antibacterial activity, the zone of inhibition was changed depending on concentration of the extract and from the stain to another, and *E. coli* strain was found to be the most sensitive. Our results offer scientific proof for the traditional utilization of this plant.

Author Contributions

N.E.A. and **K.K**.: Conceptualization; methodology. **A.B**. and **K.K**.: Software. **A.B**.: Validation. **D.A**.: Formal analysis. **S.L**. and **N.A**.: Investigation, **N.E.A**. and **K.N**.: Resources. **K.K**. and **D.A**.: Data

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curation; **D.A.**, **S.L.**, and **N.A.**: Writing—original draft. **N.E.A.** and **A.B.**: Writing—review and editing. **K.N.** and **S.G.**: Visualization. **N.A.**: Supervision. **K.K.** and **S.G.**: Project administration. **F.B.**, **H.A.R.**, and **H.B.**: Funding acquisition.

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The authors have nothing to report.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Antidiabetic · Antioxidant activity · Cirsiliol · Flavonoid extract · Hyoscyamus albus L. · LC-MS analysis

[1] L. Bourebaba, G. Sullini, J. A. Mendiola, Y. Bourebaba, A. Deghima, N. Oukil, F. Bedjou, *Ind. Crops Prod.* **2016**, *89*, 316–322.
[2] M. Abdallah, S. Hervías-Parejo, A. Traves, *Front. Ecol. Evolution* **2021**, *9*, 709876.
[3] E. R. Elsharkawy, A. Eddra, E. M. M. H. A. Abdallah, *Afr. J. Biotechnol.* **2018**, *17*, 311–321.
[4] H. Tlili, N. Hanen, A. Ben Arfa, M. Neffati, A. Boubakri, D. Buonocore, *PLoSONE* **2019**, *14*, e0213049.
[5] M. Pakkirisamy, S. K. Kalakandan, K. Ravichandran, *Pharmacog. J.* **2017**, *9*, 952–956.
[6] S. Bajkacz, J. Adamek, *Food Analytical Methods* **2018**, *11*,1330–1344.
[7] A. Bouzana, Z. Chekroud, I. Becheker, F. Kamah, N. Sakhraoui, C. Bensouici, F. Boufahja, S. A. Alsalamah, M. I. Alghonaim, S. Garzoli, H. Bendif, *Pharmaceuticals* **2025**, *18*, 578.
[8] O. R. Alara, N. H. Abdurahman, O. A. Olalere, *J. King Saud Univ. Sci.* **2017**, *1018–3647*, 30684–5.
[9] J. O. Chaves,, M. C. de Souza,, L. C. da Silva, D. Lachos-Perez, P. C. Torres-Mayanga, A. Machado, A. P. da Fonseca Machado, T. Forster-Carneiro, M. Vázquez-Espinosa, A. V. González-de-Peredo, G. F. Barbero, M. A. Rostagno, *Front. Chem* **2020**, *8*, 507887.
[10] D. Jain, S. Bisht, A. Parvez, K. Singh, P. Bhaskar, G. Koubouris, *Agriculture* **2024**, *14*, 796.
[11] A. N. Panche, A. D. Diwan, S. R. Chandra, *J. Nutr. Sci.* **2016**, *5*, 1–15.
[12] R. Orfali, S. Perveen, N. A. Siddiqui, P. Alam, T. A. Alhowiriny, A. M. Al-Taweel, S. Al-Yahya, F. Ameen, N. Majrashi, K. Alluhayb, B. Alghanem, H. Shaibah, S. I. Khan, *J. Anal. Methods Chem.* **2019**, *8*, 7435909.
[13] A. Ullah, S. Munir, S. Badshah, N. Khan, L. Ghani, B. Poulson, L. Ghani, B. G. Poulson, A. Emwas, M. Jaremko, *Molecules* **2020**, *25*, 5243.
[14] S. Yu, J. Zhong, X. Ma, L. Qiu, *Interdiscip. Medic.* **2025**, *3*, e20240077.
[15] S. Shah, K. Bashir, H. Rasheed, J. Rahman, M. Ikram, A. Shah, J. Ur Rahman, M. Ikram, A. J. Shah, K. A. Majrashi, S. M. Alnasser, F. Menaa, T. Khan, *Molecules* **2022**, *27*, 27249012.
[16] M. Akbaba, A. Isır, *Diagnostics* **2025**, *15*, 269.
[17] W. N. Nsofor, R. N. Nwaoguikpe, F. N. Ujowundu, C. O. Keke, M. T. Uba, C. V. P. Edom, *J. Drug Delivery Ther.* **2023**, *13*, 61–69.
[18] J. B. Harborne, *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed., Springer **1998**.
[19] M. R. Sahoo, S. U. Marakanam, *Pharmacogn. Res.* **2023**, *15*, 163–167.
[20] P. Lai, K. Y. Li, C. Lu, H. H. Chen, *Food Chem.* **2009**,*117*, 538–544.
[21] M. Oyaizu, *Japonaise J. Nutri. Diet.* **1986**, *44*, 307–315.
[22] G. Zengin, S. Uysal, R. Ceylan, A. Aktumsek, *Ind. Crops Prod.* **2015**, *70*, 1–6.
[23] R. J. Ruch, S. J. Cheng, J. E. Klaunig, *Carcinogenesis* **1989**, *10*, 1003–1008.
[24] Y. Mizushima, M. Kobayashi, *J. Pharm. Pharm. Res.* **1968**, *20*, 169–73.
[25] V. P. Cirillo, P. O. Wilkins, J. Anton, *J. Bacteriol.* **1963**, *86*, 1259–1264.
[26] A. Reshma, K. P. Brindha, *Asian J. Pharm. and Clin. Res.* **2014**, *7*, 121-129
[27] Y. P. Lim, S. Pang, M. M. Yusoff, S. K. Abdul Mudalip, J. Gimbun, *J. Appl. Res. Med. Aromat. Plants* **2019**, *14*, 100224.
[28] E. Al-Shalabi, A. Muzn, S. Suhair, *J. Drug Delivery Sci. Techn.* **2020**, *56*, 101544.
[29] L. Carlini, G. Tancreda, V. Iobbi, F. Caicci, S. Bruno, A. Esposito, D. Calzia, S. Benini, A. Bisio, L. Manni, A. Schito, C. E. Traverso, S. Ravera, I. Panfoli, *Cells* **2022**, *11*, 3169.
[30] J. N. Ma, C. M. Ma, In *Coffee in Health and Disease Prevention* **2015**, Elsevier Inc, pp. 635.
[31] J. Bai, Y. Wu, K. Zhong, K. Xiao, L. Liu, Y. Huang, Z. Wang, H. Gao, *J. Food Prot.* **2018**, *81*, 1187–1192.
[32] C. Srinivasulu, M. Ramgopal, G. Ramanjaneyulu, C. M. Anuradha, K. C. Suresh, *Biomed. Pharmacother.* **2018**, *108*, 547–557.
[33] H. I. El-Askary, S. S. Mohamed, H. M. A. El-Gohari, S. M. Ezzat, M. R. Meselhy, *S. Afri. J. Bot.* **2020**, *128*, 200.
[34] L. Lu, Y. Zhao, G. Yi, M. Li, L. Liao, C. Yang, C. Cho, B. Zhang, J. Zhu, K. Zou, Q. Cheng, *Chin. Med.* **2021**, *16*, 72.
[35] S. Ayari-Guentri, N. Djemouai, S. Saad, S. Karoune, R. Gaceb-Terrak, F. Rahmania, *Eur. J. Biol. Res.* **2022**, *12*, 190–206.
[36] A. N. Demirci, N. Çömlekcioğlu, A. Aygan, *Turkish J. Agriculture–Food Sci. Technol.* **2020**, *8*, 1747–1754.
[37] Z. Kebaili, S. Hameurlaine, O. Fellah, M. Djermane, N. Gherraf, A. Zellagui, *J. Biochem. Technol.* **2019**, *10*, 1–6.
[38] T. Belwal, S. M. Ezzat, L. Rastrelli, I. D. Bhatt, M. Daglia, A. Baldi, H. P. Devkota, I. E. Orhan, J. K. Patra, G. Das, C. Anandharamakrishnan, L. Gomez-Gomez, S. F. Nabavi, S. M. Nabavi, A. G. Atanasov, *Trends. Anal. Chem.* **2018**, *100*, 82–102.
[39] A. I. Dirar, D. H. M. Alsaadi, M. Wada, M. A. Mohamed, T. Watanabe, H. P. Devkota, *S. Afr. J. Bot.* **2019**, *120*, 261–267.
[40] I. Bakhouche, T. Aliat, T. Boubellouta, L. Gali, A. Sen, Y. Bellik, *S. Afr. J. Bot.* **2021**, *139*, 42–49.
[41] B. Nazneen, E. G. Wesely, M. Johnson, *Int. J. pharm. Pharm. Sci.* **2012**, *4*, 293–296.
[42] S. Palacio, M. Aitkenhead, A. Escudero, G. Montserrat-Martı, M. Maestro, *PLoS One* **2014**, *9*, 107285.
[43] A. A. Yelmate, *Eur. J. Mole. Clin. Med.* **2020**, *7*. 4362.
[44] S. Farooq, G. Shaheen, H. M. Asif, M. R. Aslam, R. Zahid, S. R. Rajpoot, S. Jabbar, F. Zafar, *Int. J.* **2022**, *20*, 15593258211069720.
[45] A. Saleem, M. Afzal, M. Naveed, S. I. Makhdoom, M. Mazhar, T. Aziz, A. A. Khan, Z. Kamal, M. Shahzad, M. Alharbi, A. Alshammari, *Molecules* **2022**, *27*, 8512.
[46] G. Shaheen, A. Ashfaq, T. Shamim, H. M. Asif, A. Ali, S. Rehman, L. Sumreen, *An Int. J.* **2022**, *20*, 15593258221126832.
[47] Z. H. Rana, M. K. Alam, M. Akhtaruzzaman, *Antioxidants* **2019**, *8*, 203.
[48] H. Edziri, O. Haddad, D. Saidana, S. Chouchen, F. Skhiri, M. Mastouri, G. Flamini, *Environ. Sci. Pollut. Res.* **2020**, *27*, 17063–17071.
[49] R. R. Ibrahim, H. A. Ibrahim, S. S. Shabana, D. GEl-Hosari, S. A. Ali, S. Mahgoub, F. A. I. Moharram, *Nat Prod. Res.* **2020**, *35*, 5183–5191.
[50] P. K. Shukla, A. Misra, B. Kumar, A. Niranjan, S. Srivastava, *Indian J. Pharm. Educ. Res.* **2020**, *54*, 368–373.
[51] S. The, A. Bekhit, J. Birch, *Antioxidants* **2014**, *3*, 67–80.
[52] S. Hachani, C. Hamia;S. Boukhalkhal, A. M. S. Silva, A. Djeridane, M. Yousfi, *Phoenix dactylifera L.* **2018**, *2352–3646*, 30038.
[53] A. Barchan, M. Bakkali, A. Arakrak, R. Pagán, A. Laglaoui, *Int. J. Curr. Microbiol. Appl. Sci.* **2014**, *3*, 399–412.
[54] N. S. F. Djuichou, E. G. Tsafack,, M. Mbiantcha, A. Gilbert, A. D. Atsamo, N. W. Yousseu, V. M. Marthe Mba, C. F. Adjouzem, *Evid Based Complement Alternat. Med.* **2019**, *2019*, 3612481.
[55] Alamgeer, A. M Uttra, U. H. Hasan, *BMC Complementary. Alternat. Med.* **2017**, *17*, 371.
[56] P. Kiran, M. M. Denni, Daniel, *Indian J. Applied Res.* **2014**, *4* 2249–555.
[57] G. G. Wang, X. H. Lu, L. X. Wei, Zhao, Z. Cui, *Evid. Based Complementary Altern. Med.* **2011**, *7*, 323171.
[58] D. J. Den Hartogh, E. Tsiani, *Biomolecules* **2019**, *9*, 99.
[59] H. Alkhalidy, W. Moore, Y. Zhang, R. McMillan, A. Wang, M. Ali, K. S. Suh, W. Zhen, Z. Cheng, Z. Jia, M. Hulver, D. Liu, *J. Diabetes Res.* **2015**, *2015*, 532984.

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[60] R. Pitchaipillai, T. Ponniah, *Int. Biol. Biomed. J.* **2016**, *2*, 4.
[61] B. Salehi, A. Ata, N. V. Anil Kumar, F. Sharopov, K. Ramírez-Alarcón, A. Ruiz-Ortega, *Biomolecules* **2019**, *9*, 551.
[62] K. D. P. P. Gunathilake, K. K. D. S. Ranaweera, H. P. Vasantha Rupasinghe, *Biomedicines* **2018**, *6*, 107.
[63] A. Cherbal, M. Bouabdallah, M. Benhalla, S. hireche, R. Desdous, *Prev. Nutr. Food Sci.* **2023**, *28*, 141–148.
[64] D. K. Patel, *Curr. Chin. Sci.* **2021**, *1*, 629–638.
[65] M. Leal, M. Mercado, M. M. Alejandra, J. M. C. Javier, I. C. Zampini, I. P. Graciela, J. Mario, I. Simirgiotis, *Heliyon* **2023**, *9*, e15276.
[66] D. M. Ghasemi, Z. Lorigooini, K. H. Amini, G. M. Sabzevary, K. M. Rafieian, *Immunol. Inflammation* **2023**, *11*, e926.
[67] M. Lavanyaa, R. K. moorthy, M. A. Alshuniaber, A. Manoharadas, *J. King Saud Univ., Sci.* **2023**, *35*, 102909.
[68] K. Kadi, A. Yahia, S. Hamli, L. Audiane, H. Khabthane, W. K. Ali, *Pak. J. Biol. Sci.* **2013**, *16*, 984–990.
[69] B. Dulger, B. S. Goncu, F. Gucin, *Asian J. Chem.* **2010**, *22*, 6879–6883.
[70] M. Vijayasanthi, V. Kannan, R. Venkataswamy, A. Doss, *J. Drugs. Med.* **2012**, *4*, 91–96.

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