

Phytochemical Composition, Antioxidant, Antifungal and Biological Activities of *Phlomis Olivieri* Benth. Grows Wildly in Sulaymaniyah–Iraq.

F. M. QADER¹ , T. A. AL-NASHI² , Z. S. LAZIM¹ 

¹Department of Horticulture, College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region, Iraq.

²Department of Food Science and Quality Control, College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region, Iraq.

ABSTRACT

This study was aimed to investigate the phytochemical contents, antioxidant, antifungal and biological activity of leaves and flowers of *Phlomis olivieri* Benth, which were collected from Qaradagh at Sulaimanyah province, during May 2021 at flowering stage. The results showed that the highest content of total phenolic and flavonoid contents, essential oil yield and antioxidant activity were found in the leaves compared with the flowers. HPLC analysis of phenolic compounds showed that *P. olivieri* leaves had the highest concentrations of Gallic acid, Apigenin, Rutin, Coumaric acid, Kampferol and Luteolin compared with the flowers and the predominant compound was Gallic acid in both plant parts. According to the GC analysis of essential oils indicated that the highest values of (Germacrene D, α -pinene, Myrcene, β -selinene, Caryophyllene, and Linalool) were in leaves and the major compound was Germacrene D in both plant parts. *Phlomis olivieri* leaves ethanolic extract have a protective effect against Aflatoxin B1 (AFB1) toxicity and ensure cellular protection of hepatic tissues in rats.

Keywords: Aflatoxins, Antioxidant activity, Essential oil, Phenolic compounds, *Phlomis olivieri*



Copyright© 2025. The Author (s). Published by College of Agricultural Engineering Sciences, University of Baghdad. This is an open-access article distributed under the term of the Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cite.

Received: 15/1/2023, Accepted: 16/4/2023, Published: 30/9/2026

INTRODUCTION

Herbal medicinal plants contain various types of novel and unique substances to treat infectious and chronic diseases. Therefore, a diet rich in antioxidants is important. Nearly 80% of people depend on traditional medicines, most of which are of plant origin. Secondary metabolic products such as tannins, terpenoids, flavonoids and alkaloids, which are obtained from plants with a wide variety of structures, have strong antimicrobial effects (Shafiq et al., 2020). In the family Lamiaceae, the genus *Phlomis* belongs to the group of medicinal genera. 100 species were used to represent it globally (Aghakhani Kaaji and Kharazian, 2019). In Iraq, there are about (6-9) species of the genus, especially in the northern regions of the country (Hasson et al., 2020). Terpenoids, flavonoids, lignans, saponins, alkaloids, phenylpropanoids, iridoids,

phenylethanoids, essential oils, and other components are among the major components of the phytochemical compounds of the genus *Phlomis* that have been studied (Tarhan et al., 2022). As many researchers reported, *Phlomis* species have unique aromatic and medicinal properties. Its flower parts are typically consumed in traditional medicine as herbal teas for a variety of purposes due to their antiulcerogenic, antiallergic, antioxidant, anti-inflammatory, antinociceptive, antimicrobial, and cytotoxic activities. This is according to information from ethnopharmacological field experiences (Sarikurkcu et al., 2014; Tarhan et al., 2022). Numerous biological and phytochemical studies have examined the various extracts obtained from *P. olivieri* aerial parts (Mohammadifar et al., 2015). A review of the literature revealed that sesquiterpene hydrocarbons, particularly

germacrene D, are the primary group of chemical constituents in some studies on the essential oil compositions of this species (Delnavazi et al., 2016). According to a research, the essential oils of *P. persica* and *P. olivieri* are sesquiterpene-rich essential oils, especially germacrene D and caryophyllene, with moderate toxic activity in the BSL test (Mohammadifar et al., 2015). Contamination of food and feed by toxigenic fungi is of serious health concern. These toxigenic fungi belong to the genera *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, ...etc. They produced mycotoxins have adverse effects on humans and animals even at low concentrations (Adekoya et al., 2019; Perincherry et al., 2019; Venkatesh and Keller, 2019). The aim of this study is to investigate the phytochemical constituents and antioxidant activity of *Phlomis olivieri* Benth and then choosing the plant part that contains the higher active constituents and antioxidant activities for studying the antifungal and biological activity of the ethanolic extract against aflatoxicosis induced by AFB1 in livers of rats.

MATERIALS AND METHODS

Collection, preparation and extraction of *Phlomis olivieri*: *Phlomis olivieri* Benth plants were collected from Qaradagh in Sulaimaniyah province at an altitude 787m during May 2021, and the plants were identified by Botanist, one of the duplicates was sent to a Botanist in the (National Herbarium of Iraq, Directorate of Seed Testing and Certification, Ministry of Agriculture, Republic of Iraq), the plant parts were washed then air-dried at room temperature, and the flowers and leaves of the plants were separated from the stem and powdered. Then, the powdered plant was prepared for further analyses. Samples were prepared from 1 g of *Phlomis olivieri* leaves and flowers powder, dissolved in 15 mL of ethanol solution (80%), shaking every 15 minutes and placed in a water bath for about 2 hr. at 38 °C and left for 22 hr. at room temperature, at the end, the solution of the extract was filtered and kept in the freezer at -18 °C until used for phytochemical analysis.

Estimation of total phenols: Folin-Ciocalteu used for determined total phenols and this

method according to the procedure previously described by Rodrigues et al (2019) with some modifications. For each ethanolic extract 100 µL was mixed with 4 mL of Folin-Ciocalteu (10%) reagent, at room temperature for 5 minutes. Then 2 mL of Na₂CO₃ (20%) was added, then the mixture in the dark was left for 60 minutes at 38 °C. To detect the absorbance using an ELISA plate reader at 765 nm. The quantity was determined concerning the standard of gallic acid in various concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg/mL). The total phenolic compound content was determined and expressed as milligrams of gallic acid equivalent (GAE) per gram of dry sample weight using a standard curve made with gallic acid.

Estimation of total flavonoids: By using aluminium chloride with the help of the colorimetric method, the total flavonoid content was obtained (Hassan et al., 2020). In such a method, about 0.5 mL of *Phlomis olivieri* extract was added to a 1.5 mL solution of ethanol (80%), followed by the addition of 0.1 mL of 10% aluminium chloride (AlCl₃), potassium acetate (1M) about 0.1 mL, and 2.8 mL of distilled water, for at least 45 minutes and left at room temperature, then 0.5 mL of water was mixed with the same amount of chemicals in the previous step as a blank. An ELISA plate reader was used to read the absorbance at 415 nm. Here, Quantification of the standard of quercetin was performed at several concentrations (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 mg/mL). Quercetin was used to create a standard curve for the calculation of the total flavonoid content, which was expressed as milligrams of Quercetin equivalent per gram of sample dry weight.

Extraction and isolation of phenolic compounds by HPLC: The phenolic compounds from a homogenized plant sample (3 g) were extracted and using ethanol/water (70/30) as solvent. Smith-Kline Company's (USA) Ultrasonic Bath was used for the extraction procedure, which took place for an hour at room temperature. The extraction yield was calculated using 5 mL of liquid extract after filtration. The solvent was removed using

a rotary evaporator under vacuum (Devarot, Elektromedicina, Ljubljana, Slovenia), and dried at 38°C to constant mass. For protection against oxidation until analysis, the dry extracts were stored in glass bottles at 4 °C. Reversed-phase HPLC analysis with a SYKAMN, Germany, was used to quantify individual phenolic compounds. HPLC chromatographic system with a Zorbax Eclipse Plus-C18-OSD, 25 cm, 4.6 mm column, Chemstation, and UV detector. The temperature of column was 30 °C, the gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was performed as follows: initial 0-4 min, 40% B; 4-10 min, 50% B; and flow-rate of 0.7 mL/min. The autosampler was used to automatically samples 100 µL of the standard and 100 µL of the injected volume of samples. The spectra were obtained in the 280 nm range (Radovanović et al., 2015).

Extraction and isolation of essential oils by GC:

The extraction of oils and GC analysis were performed according to Hcini et al., (2013). In the extraction oil, about 20 g of the dry weight of each plant part was placed in a 2-litre flask containing distilled water (1000 mL). The Clevenger set was placed under optimal operating conditions for 3 hours. After collecting the essential oil, and 20 mL of hexane was added to separate the oil from the water droplets that had also been collected. Until the analysis, the oil was gathered and kept in the fridge. A gas chromatograph (GC), Model Shimadzu 2010, Japanese origin, was used to analyses samples of 0.1 mL using capillary gas chromatography. The GC was outfitted with a flame ionization detector (FID), a 30 m×0.25 mm column, and a 0.25 mm film thickness. The injector and the FID were maintained at 340 and 280 °C, respectively. Nitrogen was used as carrier gas at a rate of 100 KPa. while regarding the temperature of the separation, the column from 100 to 300 °C at a rate of 10 °C/min was done.

Antioxidant Activity: The evaluation of antioxidant activity was conducted by the method of (Shimada et al.,1992) with some modification, after dissolving 2.4 mg of DPPH in 100 mL methanol (95%), then 10 mL of the *Phlomis* extracts were mixed with 3.995 of

methanolic DPPH, the mixture was shaken and in a dark place protected at room temperature for 30 minutes. A blank was prepared by mixing 5 mL of methanol (95%) with 3.995 mL DPPH. The absorbance in the mixture was determined by using ELISA at 515 nm. By applying the following formula to the date of absorbances, the inhibition percentage of the samples was calculated: % inhibition = [(Absorbance of control - Absorbance of the sample) / Absorbance of control] × 100.

Growth of *A. flavus* MN809141.1: The toxigenic *A. flavus* was obtained from Mycotoxin Research Laboratory, College of Agricultural Engineering Science, University of Baghdad. It was re-cultured on Potato Dextrose Agar (PDA) and incubated for 7 days at 25 ± 2 °C in an incubator (Mettler-Germany). *A. flavus* cultures were grown on PDA slants and stored at 4°C (Gallenkamp-England) for experimental works.

Growth Inhibition of *Aspergillus flavus* MN809141.1:

The growth inhibition was determined on PDA plates supplemented with different concentrations of *Phlomis olivieri* extract (2500, 5000, 7500 and 10000 µg/mL), and a 5-mm-diameter mycelial plug of the young culture of *A. flavus* MN809141.1 was transferred aseptically to the center of supplemental PDA dishes. A PDA dish without plant extract served as negative control, while (Voriconazole 10 µg/mL) was used as positive control and assayed under the same conditions, then incubated at 25 °C for 5 days. Radial growth measurements were determined when the mycelium of negative control reached the edge of the plate. All treatments were performed in triplicates in this study (Šušćević, J et al., 2021). The fungal growth inhibition was calculated as follows: $I = (C - T) / C \times 100$, where I is the mycelia growth inhibition, T is the growth of mycelium (mm) in the treatment plate, and C is the growth of mycelium (mm) in the negative control plate (Wonglom et al., 2019).

Biological study: A total of 30 mature male western albino rats weighing (123-210 g) in a good physical state were used in this study. The rats were maintained in plastic cages bedded with dry wood chips and kept in a

controlled environment (Temperature of 25 ± 2 °C with 12:12 hrs. photoperiod). Rats were acclimatized for one week with a control diet (Stark, 2016) and then rats were divided into 5 groups (n=6) as follows:

Group 1: fed control diet for 30 days with 0.5 mL of 5% dimethyl sulfoxide DMSO and considered as control.

Group 2: Fed a control diet and received AFB1 (100 µg/kg body weight) dissolved in 0.5 mL of 5% DMSO as a single daily dose by oral gavage for 30 days

Group 3: Fed a control diet and received AFB1 (100 µg/kg body weight) dissolved in 0.5 mL of 5% DMSO with 100 mg/kg body weight of *P. olivieri* extract given as a single daily dose via oral gavage for 30 days

Group 4: Fed a control diet and received AFB1 (100 µg/kg body weight) dissolved in 0.5 mL of 5% DMSO with 200 mg/kg body weight of *P. olivieri* extract given as a single daily dose via oral gavage for 30 days

Group 5: Fed a control diet and received AFB1 (100 µg/kg body weight) dissolved in 0.5 mL of 5% DMSO with 100 mg/kg body weight α -Tocopherol given as a single daily dose via oral gavage for 30 days

At the end of the study, the rats were fasted for 12 hours, the next morning they were placed in an anesthesia chamber with isoflurane, then via cardiac puncture technique, blood samples were collected, and then serum fractions were obtained by centrifugation at $3000 \times g$ for 15

minutes. The obtained serum fraction was analyzed for alanine aminotransferase enzyme (ALT) activity according to the instructions of (Sigma Aldrich assay kit). Then rats were surgically dissected wherein about (2 cm) of the hepatic tissue was obtained from each rat and immersed immediately in a fixative solution for histological study (Bano and Najam, 2019).

Statistical data analysis: The XLSTAT (Version: 2019.2.2) statistical software package, was used to perform statistical analysis, a Completely Randomized Design (CRD) was conducted for phytochemical analysis with one factor (plant part) with 5 replications. For biological study Data were submitted to the analysis of variance simple CRD (One way-ANOVA). Means were compared by Duncan's Multiple Range Test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Total phenols and Total flavonoid contents, essential oils yield and Antioxidant activity of *Phlomis olivieri* Benth : The results of the phytochemical contents of the *Phlomis olivieri* are shown in (Figure 1). The highest phenolic and flavonoid content, essential oil yield and antioxidant activity were found in the leaf extract (216.538 mg/g, 51.483 mg/g, 4.880% and 86.206%), respectively comparing to the flowers (112.349 mg/g, 15.733 mg/g, 4.057% and 71.687%), respectively.

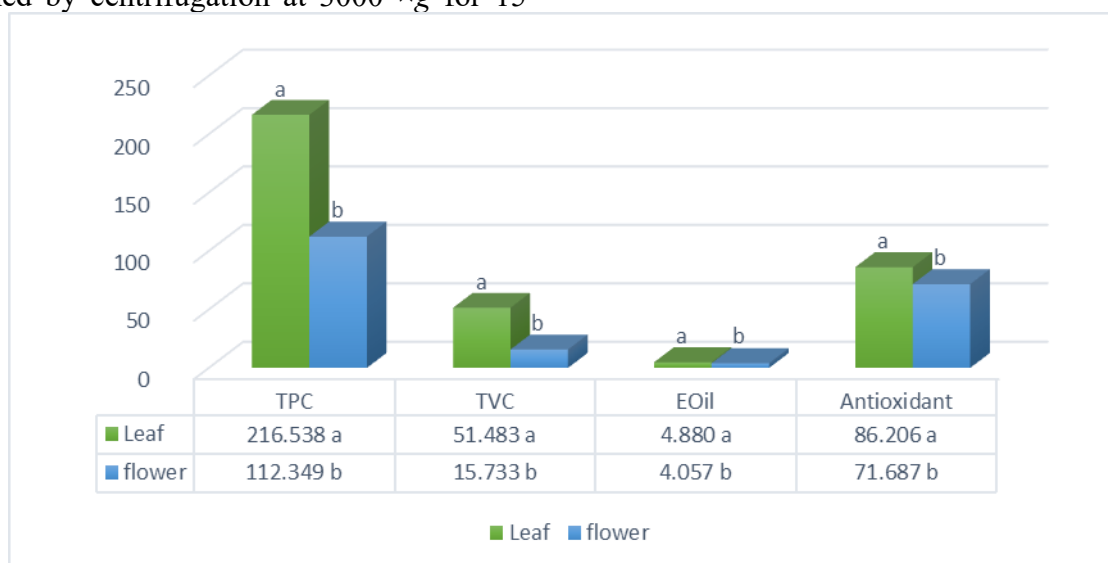


Figure 1. Total phenols and flavonoid contents (mg/g of dry sample weight), essential oil yield (%) and antioxidant activity (%), of *Phlomis olivieri* leaves and flowers extracts. *Different letters refer to significant differences between the means according to Duncan's multiple range test at $p \leq 0.05$

HPLC analysis of phenolic compounds of *Phlomis olivieri* Benth.: HPLC analysis was used to determine the separate phenolic compounds in the investigated extracts. Six phenolic compounds (Gallic acid, Apigenin, Rutin, Coumaric acid, Kaempferol and Luteolin) were detected from *Phlomis olivieri* chromatograms and quantified (Figure 2). According to the findings, the highest

concentration of these compounds were found in leaves (33.5, 14.80, 12.53, 6.66, 6.8, 3.3 ppm), respectively, compared to flowers (24.9, 10.5, 7.9, 4.8, 4.1 and 2.06 ppm), respectively. The obtained results showed that the Gallic acid had the highest concentration and Luteolin had the least concentration in both plant parts.

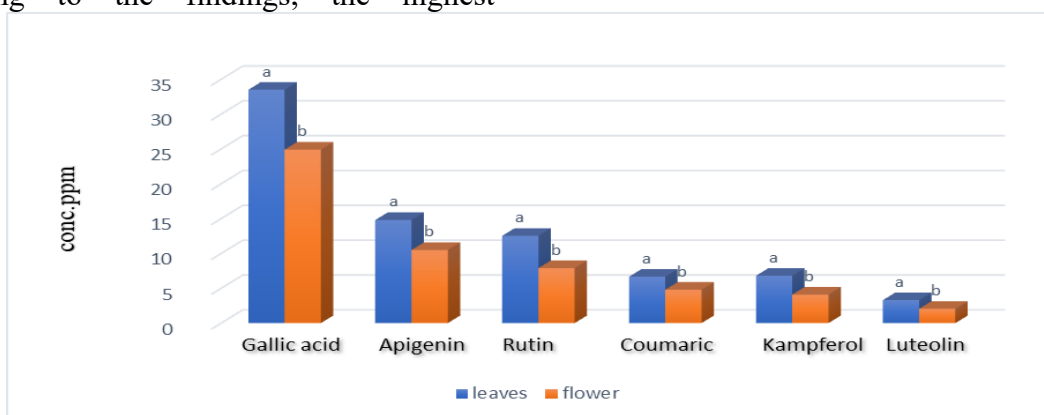


Figure 2. HPLC analysis of *Phlomis olivieri*.

*Different letters refer to significant differences between the means according to Duncan's multiple range test at $p \leq 0.05$

GC analysis of essential oils of *Phlomis olivieri* Benth.: The chemical composition of essential oil (Germacrene D, α -Pinene, Myrcene, β -Selinene, Caryophyllene and Linalool) of *P. olivieri* was presented in (Figure 3). The leaves had the highest concentrations of these compounds (55.16,

12.50, 8.23, 5, 4.1, 4 ppm), respectively comparing with flowers (29.66, 8.5, 4.8, 2.4, 3.4, 2.5 ppm), respectively. The major compound was Germacrene D in both plant parts while the lowest compound was β -Selinene in flowers.

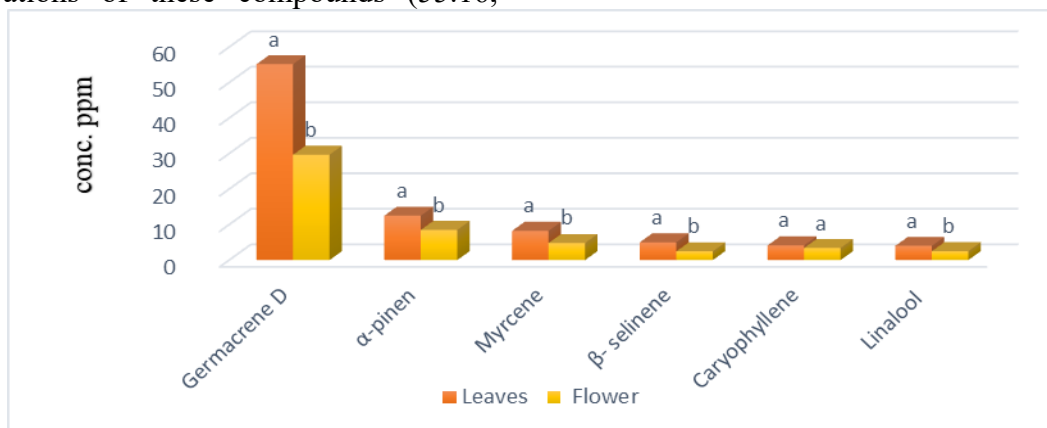


Figure 3. GC analysis of *Phlomis olivieri* essential oil

*Different letters refer to significant differences between the means according to Duncan's multiple range test at $p \leq 0.05$

The results indicate that the highest content of total phenols and total flavonoids was found in *Phlomis olivieri* leaves. The reason may be due that photosynthesis occurs in leaves, and (Shikimic acid) which is the precursor of biosynthetic of both phenolic and flavonoid are more abundant in leaves than in other organs (Saboonchian et al., 2014). Germacrene D of (55.2 %) as characterized as the main

compound was found in all oils of *P. olivieri*, also Sarkheyl et al. (2006) reported a similar compound in their study. While Mohammadifar et al. (2015) demonstrated that earlier research on the chemical makeup of this species from various areas of Iran discovered that the relative percentages of germacrene D in plants collected from Damavand, Taleghan, Chalus, and Kojour

were 28.1, 26.4, 66.1 and 48.0-58.0%, respectively. However, in the Mohammadifar et al. (2015) study, the primary compound of the essential oil of this species was identified to β -caryophyllene (25.7%), which was found in aerial parts that were collected in the Borujen area from west of Iran. In a research on *P. olivieri* essential oils, caryophyllene was found at levels of 4.11% in leaves and 3.4% in flowers. Moreover, this compound has been identified as a major component α pinene of *P. olivieri* in this present study (8.5% flower), (12.46% leaves) similar amount in previous studies Tajbakhsh et al. (2007) was found. Numerous researchers have noted the high total antioxidant and free radical scavenging activities of *Phlomis* (Amor et al., 2009; Sarikurcu et al., 2014; Tarhan et al., 2022). Delnavazi et al. (2016) reported on *Phlomis olivieri* from Iran, and presented that *P. olivieri* methanol extract demonstrated antioxidant activity in the DPPH test, the MSP exhibited a considerable free radical-scavenging activity with the IC₅₀ value of $50.4 \pm 4.6 \mu\text{g/mL}$. Additionally, several *Phlomis* species from Iran, and they found that the *P. olivieri* methanol extract had the highest antioxidant activity of the tested species (Okur et al., 2021).

Table 1. Effect of different concentrations of *Phlomis olivieri* ethanolic extract in inhibiting the growth of *Aspergillus flavus* on PDA culture medium

<i>P. olivieri</i> leaves concentration ($\mu\text{g/mL}$)	2500	5000	7500	10,000
Inhibition%	50	67	73	83

Alanine amino transferase (ALT) determination in Rats: In (Table 2), group 2 showed a significant increase in serum ALT, in comparison with the other groups. This explains the effect of AFB1 upon the hepatocytes. These results are compatible with some research; it has been suggested that measuring serum enzyme activities can help diagnose toxicity in cases of aflatoxicosis before the clinical symptoms manifest. Increased ALT activities is thought to be extremely sensitive diagnostic indicators for the diagnosis of hepatitis, dysfunction or hepatocellular injury, liver lesion or bile duct obstruction, and as a result, ALT activity increases as a result of liver injury (Oğuz et al., 2000; Karaca et al., 2021). Additionally,

Antifungal activity: The *P. olivieri* ethanolic extract effect on *A. flavus* growth is shown in (Table 1). The extract notably reduced significant ($P < 0.05$) the fungal growth. The highest dosages 10,000 $\mu\text{g/mL}$ of extract inhibit the growth of *A. flavus* on PDA, the inhibition rate was 83%. While at the lowest concentration 2500 $\mu\text{g/mL}$ significant inhibition was observed 50%. These results show the strong antifungal potency of *P. olivieri* ethanolic extract in decreasing *A. flavus* growth. The effects of antifungal activity may be related to the synergistic effect of essential oils and phenolic compounds of the extract (Anžlovar et al., 2020). Additionally, prior research demonstrated that *Phlomis* spp. from various regions display antimicrobial activity against a variety of microorganisms at varying susceptibility ranges (Mohajer et al., 2005). Aboh et al. (2014) reported that the phytochemical compounds isolated from plants have been reported to have anti-fungal properties. Many cultures around the world have a long tradition of treating skin problems, including mycosis infections, with medicinal herbs (Asgarpanah et al., 2017).

when the AFB1 treated group in a different study was compared with the control group, a significant rise in plasma ALT activities was discovered. Liver enzymes activity increases in plasma are considered to be diagnostic signs of liver damage. When the liver is injured, such as when there are hepatocellular lesions and parenchymal cell necrosis, these enzymes are released into the bloodstream (Oğuz et al., 2000; Yousef et al., 2003). Group 3 and group 4 showed a reduction in serum ALT but more in group 4. This finding explains the protective effect of the *Phlomis* ethanolic extract. Group 5 also shows a mild (insignificant $P \leq 0.05$) reduction in serum ALT in comparison with groups 2, this finding gives us an idea that *Phlomis* extract is more potent than α -

Tocopherol regarding the antioxidant effect. According to the study by Sarkhail et al. (2003) the total extracts of *P. olivieri* decreased the number of acetic acid-induced writhes in rats, demonstrating antinociceptive properties. According to various reports, a number of physical and chemical agents can reduce the hepatotoxicity caused by AFB1 in varying methods. While some substances prevent AFB1 from being absorbed, bio transformed, or bound to cellular macromolecules covalently, others promote the toxins detoxification through conjugation or other unique and unidentified mechanisms.

Table 2. Alanine amino transferase concentration in rats' serum after (30) days of feeding

Group of rats	1	2	3	4	5
ALT level IU/L	38.380 bc	58.340 a	30.200 c	25. 00 c	39.20 ab

Different superscript letters (a, b and c) show significant differences between the groups ($P \leq 0.05$)

Aflatoxin B1 (AFB1) is the most potent hepatocarcinogen classified by the International Agency for Research on Cancer (IARC, 1993) as a human carcinogen. When AFs are metabolized and transformed into extremely toxic forms to cause hepatotoxicity, the liver is the primary organ targeted. The liver tissue contained numerous histopathological findings. The hepatotoxic effects of AFB1 include growth in the liver, high levels of necrotic hepatocytes, wilting and degenerative changes in hydropic degeneration (Karaca et al., 2021). Early studies in Kenya and Uganda discovered that regions with high rates of liver cancer also had high levels of aflatoxin contamination in food products, which was in line with AFB1's hepatocarcinogenic properties in laboratory tests (Rushing and Selim, 2019). In the studies shown on rats and other animals, it has been discovered that AFB1 causes changes in oxidative stress determinants in biological materials (Rotimi et al., 2019). Oxidative stress is the imbalance between protective systems and free radical production caused by oxidative stress. The excess of reactive species can damage cell lipids, proteins, and DNA, which can lead to loss of function and even cell death, leading to many diseases such as neurodegenerative diseases, autoimmune pathologies, viral infections, inflammation, and digestive system disorders (Tarhan et al., 2022). One of the reasons for the decline in

The results indicated that the plant extracts (*Phlomis olivieri*) had hepatic-protective effects on rats by causing a significant decrease in AFB1-induced serum enzyme levels. These hepatic housekeeping enzyme leaks act as a biochemical proxy for hepatocellular damage. The ability of the plant extracts to reduce serum enzyme levels indicates that the protective action may have actually taken place at a site in the chain of events leading to cell necrosis as well as at the activation steps of AFB1 required for hepatic cell injury (Shyamal et al., 2010).

antioxidant enzyme activity brought on by the oxidative stress caused by AFB1 may be the inhibition effect of ROS brought on by AFB1 on the enzyme activity. The oxidation of proteins may result from the ROS binding to proteins, which changes their structure, this could be the second explanation. According to their findings, these modifications can be directly attributed to AF's production of free radicals and the consumption of antioxidants during free radicals' transformation into benign or less harmful metabolites (Karaca et al., 2021). Antioxidants are molecules that neutralize these dangerous free radicals, slow down oxidation, and shield cells from oxidative stress (Berrani et al., 2021). The biochemical findings are compatible with the histopathology finding of the rat under study.

Histological examination: In this study, the histological results of the control group were found to have a normal histological appearance (Figure 4a), while in a group of AFB1 administration caused acute and chronic inflammation manifested as abnormal congested periportal veins and sinuses (Figure 4b). According to Abdel-Wahhab et al. (2010), rats treated with AFB1 had livers with increased bile duct proliferation and congestion in the portal area, similar to the case of this study (Figure 4c). Moreover, focal necrosis and fibrosis were observed in the livers of rats from the AFB1 group and 100 mg/kg/day of the *Phlomis* group (Figure 4d).

Vacuolar degenerative changes in hepatocytes and hydropic degeneration were observed in the livers of the rats from the AFB₁ group, these changes were determined to decrease slightly in the AFB₁ and 100 mg *Phlomis* groups. While 200 mg/kg/day of *Phlomis* was not found to degenerate and showed normal histological results (Figure 4e). Thus, *Phlomis* extract affected to decrease and disappear AFB₁ on liver tissue. As a result, groups of

AFB₁ with α -Tocopherol and control were compared (Figure 4f) group 5 shows focal fibrosis. *Phlomis olivieri* extracts have been shown to contain phenolic compounds that are associated with the antioxidant activity due to their redox properties, which can be important in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. (Miller et al., 2000).

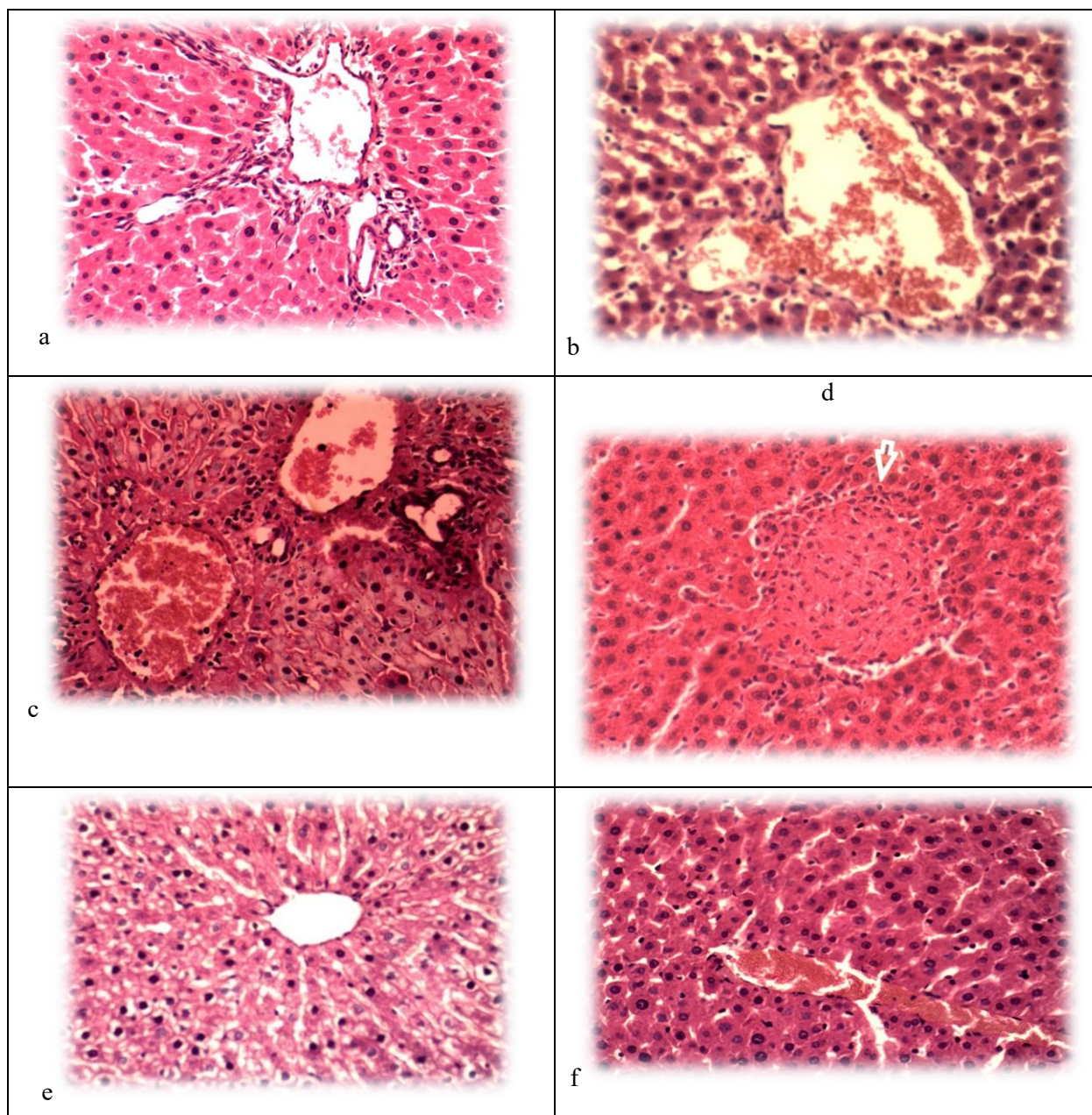


Figure 4. Histological evidence for the protective effect of ethanolic extracts on aflatoxin-induced hepatic damage in rats. (a) Control group, normal histological appearance. (b) AFB₁ receiving group, abnormal portal, congestion vein and sinuses. (c) AFB₁ with 100 mg/kg of body weight of *P. olivieri* extract, portal area congestion and increase bile duct. (d) AFB₁ with 100 mg/kg of body weight of *P. olivieri* extract, focal fibrosis. (e) AFB₁ with 200 mg/kg of body weight of *P. olivieri* extract, normal histological appearance (f) AFB₁ with α -Tocopherol group with focal fibrosis.

Antioxidants are substances that, when present in small amounts compared to an oxidizable substrate, can significantly slow down or prevent that substrate from oxidizing. Polyphenols in particular are known to reduce oxidative stress among phytochemical components. Phenolic compounds are secondary metabolites that are identified to be responsible for the antioxidant activity of plants (Lazeeza, 2021). It is well known that phenolic compounds detoxify free radicals and donate hydrogen to reduce oxidative damage. It is hypothesized that these substances help explain the benefits to health (Radovanović et al., 2015). Due to their antioxidant properties, phenolic compounds inhibit the production of reactive oxygen and nitrogen species (ROS and RNS) in living cells by inhibiting the production of free radicals, radical chain reactions, certain enzymes, and chelate-forming metal ions (Mutha et al., 2021); (Tarhan et al., 2022). According to research by Abdel-Wahhab et al. (2010), the administration of AFB1 resulted in a vacuum and mononuclear cellular infiltration among hepatocytes, which in turn reduced protein reaction in abnormally vacuolated nucleated hepatocytes and resulted in the loss of the regular chromatin pattern. Additionally, the glycogen rosette, endoplasmic profile, and small fatty droplets were lost. Rats receiving AFB1 treatment had livers with periportal hepatocellular degeneration and significant biliary proliferation. Furthermore, Karaca et al. (2021) demonstrated that AFB1 significantly altered the histopathology of liver tissues. The structure of phenolic compounds, particularly the benzene ring and position and the number of the OH groups, determines their ability or potency to have an antioxidant function. Antioxidant molecules are stabilized as a result of their reaction with free radicals, to the benzene ring. Gallic acid was found to be the primary phenolic component of the *Phlomis olivieri* extract according to our study. Gallic acid is a phenolic acid with one carboxylic acid and three hydroxyls group. However, the hydroxyl group acts as an antioxidant by creating gallic acid-free radicals (Rajan and Muraleedharan, 2017). The findings of the present study demonstrated that the

pretreatment of rats with plant extracts (*P. olivieri*) resulted in a hepatoprotective effects against AFB1 toxicity. Vacuolar degenerative changes of hepatocytes and hydropic degeneration in addition to focal hepatic necrosis with fibrosis were observed in the AFB1 group, these changes were slightly decreased in the AFB1 group that were treated with 100 mg/kg of body weight *P. olivieri* extract, while those group that was treated with 200 mg/kg body weight of *P. olivieri* extract exhibit nearly normal histopathological examination. In consequence, the histopathological results are totally compatible with biochemical results.

CONCLUSIONS

Leaves ethanolic extract of *Phlomis olivieri* was found to be high in total phenolic and flavonoid contents, essential oil yield and high antioxidant activity. The ethanolic extract had powerful effect on *A. flavus* growth. The ethanolic extract have hepatoprotective effects against AFB1 toxicity in rats.

ACKNOWLEDGMENTS

We thank Dr Halema Al-Bahadli (College of Agricultural Engineering Science, University of Baghdad) for supplying the isolate of *A. flavus*.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

AUTHOR'S CONTRIBUTION STATEMENT

Author 1 (Frmesk Mohammed Qader): As the MSc student, was principally responsible for conducting the study. **Author 2 (Taghreed Abid Wahwah Al-Nashi):** Supervised the MSc research and was principally responsible for the biological part of the study. **Author 3 (Zainab Sabah Lazim):** Supervised the MSc research and was principally responsible for the plant-related components. Both supervisors provided academic guidance, scientific direction, and oversight throughout the study. All authors contributed to the interpretation of the results, reviewed and approved the final manuscript, and agreed to be accountable for the work.

REFERENCES

Abdel-Wahhab, M. A., Hassan, N. S., El-Kady, A. A., Khadrawy, Y. A., El-Nekeety, A. A., Mohamed, S. R., & Mannaa, F. A. Abdel-

- Wahhab, M. A., Hassan, N. S., El-Kady, A. A., Khadrawy, Y. A., El-Nekeety, A. A., Mohamed, S. R., ... & Mannaa, F. A. (2010). Red ginseng extract protects against aflatoxin B1 and fumonisins-induced hepatic pre-cancerous lesions in rats. *Food and Chemical Toxicology*, 48(2), 733–742.
- Aboh, M. I., Olayinka, B. O., Adeshina, G. O., & Oladosu, P. (2014). Antifungal Activities of Phyto Compounds from *Mitracarpus villosus* (Sw.) DC from Abuja, Nigeria. *Journal of Microbiology Research*, 4(2), 86–91. <https://doi.org/10.5923/j.microbiology.20140402.07>
- Adekoya, I., Njobeh, P., Obadina, A., Landschoot, S., Audenaert, K., Okoth, S., De Boevre, M., & De Saeger, S. (2019). Investigation of the metabolic profile and toxigenic variability of fungal species occurring in fermented foods and beverage from nigeria and South Africa using UPLC-MS/MS. *Toxins*, 11(2). <https://doi.org/10.3390/toxins11020085>
- Aghakhani Kaaji, F., & Kharazian, N. (2019). Flavonoid Diversity and Morphological Variations Among Seven *Phlomis* Species in Zagros, Iran. *Iranian Journal of Science and Technology, Transaction A: Science*, 43(2), 415–431. <https://doi.org/10.1007/s40995-017-0344-4>
- Amor, I. L. Ben, Boubaker, J., Sgaier, M. Ben, Skandrani, I., Bhouri, W., Neffati, A., Kilani, S., Bouhlel, I., Ghedira, K., & Chekir-Ghedira, L. (2009). Phytochemistry and biological activities of *Phlomis* species. *Journal of Ethnopharmacology*, 125(2), 183–202. <https://doi.org/10.1016/j.jep.2009.06.022>
- Anžlovar, S., Janeš, D., & Koce, J. D. (2020). The effect of extracts and essential oil from invasive *Solidago* spp. and *Fallopia japonica* on crop-borne fungi and wheat germination. *Food Technology and Biotechnology*, 58(3), 273–283. <https://doi.org/10.17113/ftb.58.03.20.6635>
- Asgarpanah, J., Hashemi, S. J., Hashemi, E., & Askari, K. (2017). In vitro antifungal activity of some traditional Persian medicinal plants on pathogenic fungi. *Chinese Journal of Integrative Medicine*, 23(6), 433–437. <https://doi.org/10.1007/s11655-015-2181-7>
- Bano, N., & Najam, R. (2019). Experimental research Histopathological and biochemical assessment of liver damage in albino Wistar rats treated with cytotoxic platinum compounds in combination with 5-fluorouracil.
- Berrani, A., Marmouzi, I., Bouyahya, A., Kharbach, M., El Hamdani, M., El Jemli, M., Lrhorfi, A., Zouarhi, M., Faouzi, M. E. A., & Bengueddour, R. (2021). Phenolic Compound Analysis and Pharmacological Screening of *Vitex agnus-castus* Functional Parts. *BioMed Research International*, 2021. <https://doi.org/10.1155/2021/6695311>
- Delnavazi, M. R., Mohammadifar, F., Rustaie, A., Aghaahmadi, M., & Yassa, N. (2016). Phytochemical constituents , antioxidant activity and toxicity potential of *Phlomis olivieri* Benth. *Research Journal of Pharmacognosy*, 3(2), 9–15.
- Hassan, A., Akmal, Z., Khan, N., & Alparslan, Y. (2020). The Phytochemical Screening and Antioxidants Potential of *Schoenoplectus triqueter* L. Palla. *Journal of Chemistry*, 2020. <https://doi.org/10.1155/2020/3865139>
- Hasson, S. M., AL-Qaraawi, H. A. H., & Hussein, R. H. (2020). Taxonomic study of the genus *phlomis* L. (lamiaceae) in Iraq based on trichome and palynological characters. *Journal of Global Pharma Technology*, 12(6), 151–158.
- Hcini, K., Sotomayor, J. A., Jordan, M. J., & Bouzid, S. (2013). Chemical composition of the essential oil of rosemary (*Rosmarinus officinalis* L.) of Tunisian origin. *Asian Journal of Chemistry*, 25(5), 2601–2603. <https://doi.org/10.14233/ajchem.2013.13506>
- Karaca, A., Yilmaz, S., Kaya, E., & Altun, S. (2021). The effect of lycopene on hepatotoxicity of aflatoxin B1 in rats. *Archives of Physiology and Biochemistry*, 127(5), 429–436. <https://doi.org/10.1080/13813455.2019.1648516>
- Miller, H. E., Rigellhof, F., Marquart, L., Prakash, A., & Kanter, M. (2000). Antioxidant Content of Whole Grain Breakfast Cereals, Fruits and Vegetables. *Journal of the American College of Nutrition*, 19(August), 312S-319S.

<https://doi.org/10.1080/07315724.2000.10718966>

Mohajer, M., Sarkhail, P., Hajarolasvadi, N., Zamani, M. J., Khorasani, R., Shafiee, A., ... & Abdollahi, M. (2005). Antiinflammatory and analgesic effects of *Phlomis lanceolata* Boiss. and Hohen. extracts and examination of their components. *International Journal of Pharmacology*, 2(1), 50–54.

Mohammadifar, F., Delnavazi, M. R., & Yassa, N. (2015). Chemical analysis and toxicity screening of *Phlomis olivieri* Benth. and *Phlomis persica* Boiss. essential oils. *Pharmaceutical Sciences*, 21(1), 12–17.

<https://doi.org/10.15171/PS.2015.11>

Mutha, R. E., Tatiya, A. U., & Surana, S. J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Future Journal of Pharmaceutical Sciences*, 7(1).

<https://doi.org/10.1186/s43094-020-00161-8>

Oğuz, H., Kurtoğlu, V., & Coşkun, B. (2000). Preventive efficacy of clinoptilolite in broilers during chronic aflatoxin (50 and 100 ppb) exposure. *Research in Veterinary Science*, 69(2), 197–201.

<https://doi.org/10.1053/rvsc.2000.0417>

Okur, M. E., Karadağ, A. E., Özhan, Y., Sipahi, H., Ayla, Ş., Daylan, B., Kültür, Ş., Demirci, B., & Demirci, F. (2021). Anti-inflammatory, analgesic and in vivo-in vitro wound healing potential of the *Phlomis rigida* Labill. extract. *Journal of Ethnopharmacology*, 266(September 2020).

<https://doi.org/10.1016/j.jep.2020.113408>

Perincherry L., Lalak-Kanczugowska J., S. L. (2019). Fusarium-produced mycotoxins in plant-pathogen interactions. *Toxins*, 11.

Radovanović, B., Mladenović, J., Radovanović, A., Pavlović, R., & Nikoli, V. (2015). Phenolic Composition, Antioxidant, Antimicrobial and Cytotoxic Activities of *Allium porrum* L. (Serbia) Extracts. *Journal of Food and Nutrition Research*, 3(9), 564–569.

<https://doi.org/10.12691/jfnr-3-9-1>

Rajan, V. K., & Muraleedharan, K. (2017). A computational investigation on the structure, global parameters and antioxidant capacity of a polyphenol, Gallic acid. *Food Chemistry*, 220, 93–99.

<https://doi.org/10.1016/j.foodchem.2016.09.178>

Rodrigues, A. B., De Almeida-Apolonio, A. A., Alfredo, T. M., Da Silva Dantas, F. G., Campos, J. F., Cardoso, C. A. L., De Picoli Souza, K., & De Oliveira, K. M. P. (2019). Chemical composition, antimicrobial activity, and antioxidant activity of *ocotea minarum* (Nees & Mart.) mez. *Oxidative Medicine and Cellular Longevity*, 2019.

<https://doi.org/10.1155/2019/5736919>

Rotimi, O. A., Rotimi, S. O., Goodrich, J. M., Adelani, I. B., Agbonihale, E., & Talabi, G. (2019). Time-course effects of acute aflatoxin B1 exposure on hepatic mitochondrial lipids and oxidative stress in rats. *Frontiers in Pharmacology*, 10(MAY), 1–10.

<https://doi.org/10.3389/fphar.2019.00467>

Rushing, B. R., & Selim, M. I. (2019). Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. *Food and Chemical Toxicology*, 124, 81–100.

<https://doi.org/10.1016/j.fct.2018.11.047>

Saboonchian, F., Jamei, R., & Hosseini Sarghein, S. (2014). Phenolic and flavonoid content of *Elaeagnus angustifolia* L. (leaf and flower). *Avicenna Journal of Phytomedicine*, 4(4), 231–238.

<http://www.ncbi.nlm.nih.gov/pubmed/25068137>
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4110780>

Sarikurkcü, C., Uren, M. C., Tepe, B., Cengiz, M., & Kocak, M. S. (2014). Phenolic content, enzyme inhibitory and antioxidative activity potentials of *Phlomis nissolii* and *P. pungens* var. *pungens*. *Industrial Crops and Products*, 62, 333–340.

<https://doi.org/10.1016/j.indcrop.2014.09.002>

Sarkhail, P., Abdollahi, M., & Shafiee, A. (2003). Antinociceptive effect of *Phlomis olivieri* Benth., *Phlomis anisodonta* Boiss. and *Phlomis persica* Boiss. total extracts. *Pharmacological Research*, 48(3), 263–266.

[https://doi.org/10.1016/S1043-6618\(03\)00151-8](https://doi.org/10.1016/S1043-6618(03)00151-8)
SARKHEYL, P., AMIN, G. R., & Shafiei, A. (2006). Composition of the essential oil of *Phlomis olivieri* Benth. from north of Iran. 14(2).

- Shafiq, Y., Asghar, M. A., Ali, H., Abedien, S., Rehman, A. A., & Anser, H. (2020). In Vitro Assessment of Antimicrobial Potential of Ethanolic and Aqueous Extract of Against Some Highly Resistant Pathogens. *ANNALS of Jinnah Sindh Medical University*, 6(1), 3–9.
<https://doi.org/10.46663/ajsmu.v6i1.3-9>.
- Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992) Antioxidative Properties of Xanthan on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion. *Journal of Agricultural and Food Chemistry*, 40, 945-948.
<https://doi.org/10.1021/jf00018a005>
- Shyamal, L., Latha, P. G., Suja, S. R., Shine, V. J., Anuja, G. I., Sini, S., Pradeep, S., Shikha, P., & Rajasekharan, S. (2010). Hepatoprotective effect of three herbal extracts on aflatoxin B1-intoxicated rat liver. *Singapore Medical Journal*, 51(4), 326–331.
- Stark, J. (2016). Product Data. 145–180.
https://doi.org/10.1007/978-3-319-24436-5_8
- Šušković, J., Kos, B., Goreta, J., & Matošić, S. (2001). Role of lactic acid bacteria and bifidobacteria in synbiotic effect.
- Tajbakhsh, M., Rineh, A., & Khalilzadeh, M. A. (2007). Chemical composition of the essential oils from leaves, flowers, stem and root of *Phlomis olivieri* Benth. *Journal of Essential Oil Research*, 19(6), 501–503.
- Tarhan, L., Ozturk Urek, R., Oner, A., & Nakiboglu, M. (2022). Evaluation of phenolic profiles, antioxidant activities, and cytotoxic and apoptotic potentials of *Phlomis angustissima* and *Phlomis fruticosa*, medicinal plants from Turkey. *European Journal of Integrative Medicine*, 55.
<https://doi.org/10.1016/j.eujim.2022.102188>
- Venkatesh, N., & Keller, N. P. (2019). Mycotoxins in conversation with bacteria and fungi. *Frontiers in Microbiology*, 10(MAR), 1–10.
<https://doi.org/10.3389/fmicb.2019.00403>
- Wonglom, P., Daengsuwan, W., Ito, S. ichi, & Sunpapao, A. (2019). Biological control of *Sclerotium* fruit rot of snake fruit and stem rot of lettuce by *Trichoderma* sp. T76-12/2 and the mechanisms involved. *Physiological and Molecular Plant Pathology*, 107(March), 1–7.
<https://doi.org/10.1016/j.pmpp.2019.04.007>
- Yousef, M. I., Salem, M. H., Kamel, K. I., Hassan, G. A., & El-Nouty, F. D. (2003). Influence of ascorbic acid supplementation on the haematological and clinical biochemistry parameters of male rabbits exposed to aflatoxin B1. *Journal of Environmental Science and Health*, 38(2), 193–209.

المحتوى الكيميائي والفعاليات المضادة للأكسدة وللطفريات والفعالية البايولوجية لـ *Phlomis olivieri* النامي برياً في
محافظة السليمانية /العراق

فرميسك محمد قادر¹، تغريد عبد وحواح الناشي²، زينب صباح لازم¹

¹ قسم البستنة، كلية علوم الهندسة الزراعية، جامعة السليمانية

² الصناعات الغذائية و السيطرة النوعية، كلية علوم الهندسة الزراعية، جامعة السليمانية

المستخلص

هدفت هذه الدراسة الى دراسة المحتوى الكيميائي والفعالية المضادة للأكسدة والمضادة للطفريات والفعالية البايولوجية لأوراق وأزهار *Phlomis olivieri* التي تم جمعها من قرداغ في محافظة السليمانية / إقليم كردستان العراق في ايار 2021 خلال مرحلة التزهير ، بينت النتائج أن الاوراق أعطت أعلى محتوى من الفينولات والفلافونيدات والزيوت الطيارة الكلية والفعالية المضادة للأكسدة مقارنة بالأزهار ،كما أظهرت نتائج تحليل الفينولات بتقنية ال HPLC أن أعلى التراكيز لكل من حامض الغاليك والأبيجينين والروتين وحامض الكوماريك والكامفيرول والليوتلين كان في الأوراق قياساً بالأزهار وكان حامض الغاليك هو الحامض السائد في كلا جزئي النبات، كذلك الحال بالنسبة لتحليل الزيوت الطيارة بتقنية GC أن أوراق نبات أعطت أعلى التراكيز لكل من الكرماسين-D والالفاباينين والمايرسين والبيتاسيولانين والكاريوفلين واللينالول مقارنة بالأزهار وكان مركب الكرماسين D- هو المركب السائد في كل من الأوراق والأزهار. ان المستخلص الكحولي لأوراق *Phlomis olivieri* له تأثير حافظ ضد سمية لأفلاتوكسين B₁ ويضمن حماية خلايا انسجة الكبد في الفئران.

كلمات مفتاحية : سموم الافلا ،الفعالية المضادة للاكسدة،الزيوت الطيارة،المركبات الفينولية، *Phlomis olivieri* .