

Hepatoprotective activity of algerian carob tree leaves (*Ceratonia siliqua* L.) against CCl₄-induced liver damage in mice

Atividade hepatoprotetora das folhas da alfarrobeira argelina (*Ceratonia siliqua* L.) contra danos hepáticos induzidos por CCl₄ em camundongos

Actividad hepatoprotectora de las hojas del algarrobo argelino (*Ceratonia siliqua* L.) contra el daño hepático inducido por CCl₄ en ratones

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ABSTRACT

This study investigates the phytochemical composition and biological activities of *Ceratonia siliqua* L. leaves. The research is justified by the increasing demand for natural bioactive compounds in pharmacological applications. The study aims to evaluate the antioxidant, antimicrobial, and hepatoprotective properties of methanolic and aqueous extracts of *Ceratonia siliqua* leaves. To

achieve this, phytochemical screening and quantitative assays were conducted, revealing a high abundance of bioactive compounds, particularly total polyphenols in the aqueous extract (43.55 ± 0.22 mg GAE/g). Antioxidant activity assays demonstrated significant efficacy, with the methanolic extract excelling in the DPPH test ($IC_{50} = 25.6$ μ g/ml) and the aqueous extract in the β -carotene bleaching test ($IC_{50} = 21.59$ μ g/ml). Antimicrobial tests showed broad-spectrum activity, with the aqueous extract exhibiting a notable inhibition zone against *Staphylococcus aureus* (23 mm). The hepatoprotective potential of the aqueous extract was evaluated in mice at 400 mg/kg, significantly reducing carbon tetrachloride-induced liver damage. Key liver enzymes ASAT and ALAT were markedly decreased (222.6 U/L to 26.6 U/L and 415.9 U/L to 156 U/L, respectively). These findings highlight the therapeutic potential of *Ceratonia siliqua* leaves as a natural source of antioxidant, antimicrobial, and hepatoprotective agents, suggesting promising applications in pharmacology and related fields.

Keywords: *Ceratonia siliqua*, antioxidant activity, phenolic compounds, antimicrobial activity, hepatoprotective activity

RESUMO

O presente estudo investiga a composição fitoquímica e as atividades biológicas das folhas de *Ceratonia siliqua* L. A pesquisa se justifica pela crescente demanda por compostos bioativos naturais em aplicações farmacológicas. O objetivo é avaliar as propriedades antioxidantes, antimicrobianas e hepatoprotetoras dos extratos metanólico e aquoso das folhas de *Ceratonia siliqua*. Para isso, foram realizados triagem fitoquímica e ensaios quantitativos, que revelaram uma alta abundância de compostos bioativos, com destaque para os polifenóis totais no extrato aquoso ($43,55 \pm 0,22$ mg GAE/g). Ensaios de atividade antioxidante demonstraram eficácia significativa, com o extrato metanólico se destacando no teste DPPH ($IC_{50} = 25,6$ μ g/ml) e o extrato aquoso no teste de descoloração de β -caroteno ($IC_{50} = 21,59$ μ g/ml). Os testes antimicrobianos mostraram atividade de amplo espectro, com o extrato aquoso exibindo uma zona de inibição notável contra *Staphylococcus aureus* (23 mm). O potencial hepatoprotetor do extrato aquoso foi avaliado em camundongos a 400 mg/kg, reduzindo significativamente os danos hepáticos induzidos por tetracloreto de carbono. As enzimas hepáticas ASAT e ALAT apresentaram reduções marcantes (222,6 U/L para 26,6 U/L e 415,9 U/L para 156 U/L, respectivamente). Os resultados destacam o potencial terapêutico das folhas de *Ceratonia siliqua* como fonte natural de agentes antioxidantes, antimicrobianos e hepatoprotetores, sugerindo aplicações promissoras nas áreas de farmacologia e afins.

Palavras-chave: *Ceratonia siliqua*, atividade antioxidante, compostos fenólicos, atividade antimicrobiana, potencial hepatoprotetor.

RESUMEN

El presente estudio investiga la composición fitoquímica y las actividades biológicas de las hojas de *Ceratonia siliqua* L. La investigación se justifica por la creciente demanda de compuestos bioactivos naturales en aplicaciones farmacológicas. El objetivo es evaluar las propiedades antioxidantes, antimicrobianas y hepatoprotectoras de los extractos metanólico y acuoso de las hojas de *Ceratonia siliqua*. Para ello, se realizaron pruebas fitoquímicas y ensayos cuantitativos que revelaron una alta abundancia de compuestos bioactivos, destacándose los polifenoles totales en el extracto acuoso ($43,55 \pm 0,22$ mg GAE/g). Los ensayos de actividad antioxidante demostraron una eficacia significativa, destacando el extracto metanólico en la prueba de DPPH ($IC_{50} = 25,6$ μ g/ml) y el extracto acuoso en la prueba de decoloración de β -caroteno ($IC_{50} = 21,59$ μ g/ml). Las pruebas antimicrobianas mostraron una actividad de amplio espectro, con el extracto acuoso exhibiendo una notable zona de inhibición contra *Staphylococcus aureus* (23 mm). El potencial hepatoprotector del extracto acuoso se evaluó en ratones a 400 mg/kg, reduciendo significativamente los daños

hepáticos inducidos por tetracloruro de carbono. Las enzimas hepáticas ASAT y ALAT mostraron reducciones significativas (222,6 U/L a 26,6 U/L y 415,9 U/L a 156 U/L, respectivamente). Los resultados destacan el potencial terapéutico de las hojas de *Ceratonia siliqua* como fuente natural de agentes antioxidantes, antimicrobianos y hepatoprotectores, sugiriendo aplicaciones prometedoras en farmacología y áreas relacionadas.

Palabras clave: *Ceratonia siliqua*, actividad antioxidante, compuestos fenólicos, actividad antimicrobiana, potencial hepatoprotector.

1 INTRODUCTION

Carob (*Ceratonia siliqua* L.), also known as the carob tree, is a symbolic plant of the Mediterranean regions, primarily valued for its sweet pods but also its medicinally significant leaves. Traditionally, carob leaves have been used in folk medicine to treat various ailments. Modern research has intensified the exploration of their bioactive compounds and health benefits (Battle *et al.*, 1997).

Carob leaves are rich in flavonoids, phenols, and other secondary metabolites with antioxidant, anti-inflammatory, and antimicrobial properties (Custódio *et al.*, 2011). Studies show their extracts exhibit notable antimicrobial activity against Gram-positive and Gram-negative bacteria and pathogenic fungi, suggesting potential for natural antibacterial agents (Aissani *et al.*, 2012; Al-Fawwaz *et al.*, 2016).

The liver, essential for nutrient metabolism and detoxification, is vulnerable to damage from toxins, infections, and alcohol (Bhardwaj *et al.*, 2023). Research on hepatoprotective agents, including medicinal plant extracts, is vital. Antioxidants, which neutralize oxidative stress caused by reactive oxygen species (ROS), play a key role in liver protection and combating degenerative diseases (Halliwell *et al.*, 2015). Carob leaves, rich in phenolic compounds and flavonoids, have exceptional antioxidant activity (Custódio *et al.*, 2011).

This study investigates the biological activities of *Ceratonia siliqua* L. leaves by analyzing the bioactive compounds in methanolic and aqueous extracts. The research aims to evaluate their antioxidant and antimicrobial properties, as well as their hepatoprotective potential, with a particular focus on *in vivo* experiments. The aqueous extract was selected for the hepatoprotective study based on its higher concentration of total polyphenols and superior antioxidant performance compared to the methanolic extract. The hepatoprotective activity was evaluated in a mouse model of carbon tetrachloride-induced liver damage, offering a detailed assessment of the therapeutic potential of *Ceratonia siliqua* L. leaves for pharmaceutical and nutraceutical applications.

2 METHODOLOGY

2.1 PREPARATION OF *CERATONIA SILIQUA* LEAVES POWDER (CSLP)

Ceratonia siliqua L. leaves were assembled in February 2024 from the Bocaat EL-Errari region, near EL-Karimia, in the Chlef province of Algeria. The leaves were initially cleaned to remove any impurities and then subjected to air-drying in a shaded environment. Subsequently, The dried leaves were finely ground using an electric grinder, and the resulting CSLP was stored in airtight glass jars for subsequent analysis.

2.2 PREPARATION OF AQUEOUS AND METHANOLIC EXTRACTS

The aqueous extract was prepared following (Djidel *et al.*, 2018) using cold maceration, where 25 g of CSLP was mixed with 250 ml of sterile distilled water, stirred continuously for 24 hours at room temperature, and centrifuged at 4000 rpm for 15 minutes to recover the supernatant. The methanolic extract was prepared as described by (Begashaw *et al.*, 2017), with 10 g of CSLP macerated in 100 ml of methanol, stirred for 72 hours at room temperature, and protected from light. Filtrates were concentrated via rotary evaporation at 45°C and dried to remove residual solvent. Extracts were stored at 4°C for future use.

The percentage of crude dry extract (both methanolic and aqueous) was calculated using the following formula:

$$R\% = \frac{M}{M_0} \times 100$$

where:

M = is the mass in grams of the resulting dry extract

M₀ = is the mass in grams of the used CSLP.

2.3 PHYTOCHEMICAL SCREENING AND QUANTIFICATION OF POLYPHENOLS AND FLAVONOIDS

Qualitative phytochemical analysis was conducted following the method of (Houmènou *et al.*, 2018), based on color and precipitation reactions. The method of (Yeo *et al.*, 2011) was used to detect polyphenols, revealing a blue-black, green, or dark black coloration. Quantification was performed using the Folin-Ciocalteu reagent method as described by (Kouamé *et al.*, 2021). The presence of flavonoids was indicated by a red-orange or yellow color, while a white-yellowish precipitate indicated the presence of alkaloids (María *et al.*, 2018). For flavonoid quantification, the aluminum chloride method outlined by (Kouamé *et al.*, 2021) was adopted. The Liebermann-Burchard reaction was used to identify sterols and polyterpenes, forming a red ring in the presence

of chloroform and sulfuric acid (N'Guessan *et al.*, 2009). Free quinones were detected by adding 1% NaOH, producing yellow, red, or violet colors Dohou (2015). Catechin tannins were detected by a dark green coloration, while gallic tannins produced a blue-green color BentabetLasgaa (2015). Finally, saponins were identified by persistent foam formation of 1 cm for 15 minutes Shubham (2019).

2.4 IN VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF CSLP EXTRACTS

Antioxidant activity was evaluated using the DPPH assay, FRAP assay, and β -carotene bleaching test. The DPPH assay followed (Zargoosh *et al.*, 2019), the FRAP assay (Fernandes *et al.*, 2016), and the β -carotene bleaching test (Ahmedi *et al.*, 2022). Absorbance readings were taken at 517, 700, and 470 nm for the respective assays using an OPTIZEN POP-V spectrophotometer.

The antimicrobial activity of the extracts was assessed via the disk diffusion method (Mohamed *et al.*, 2020). Target strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*) were prepared in 12-hour precultures, with absorbance at 600 nm corresponding to 10^8 CFU/mL. Mueller-Hinton agar plates were inoculated with microbial suspensions, and paper disks (6 mm) soaked in 200 mg/mL extracts were applied. DMSO disks served as negative controls, and AZTREONAM ATM 30, RIFAMPICINE RD 30, and CEPHALOTHIN KF 30 as positive controls. Plates were incubated at 37°C for 24 hours before recording results.

2.5 IN VIVO HEPATOPROTECTIVE ACTIVITY STUDY

The hepatoprotective properties and LD50 of the aqueous CSLP extract were evaluated following (Meharie *et al.*, 2020) and (Sintayehu *et al.*, 2012). The study, conducted in a university animal facility, involved mice housed under controlled conditions with a 12-hour light-dark cycle, unrestricted water, and standard feed after a week of acclimation. Mice were divided into six groups of six: (1) Negative Control received distilled water for six days), (2) Positive Control treated with silymarin for six days and CCl₄ on the fourth day), (3) Toxic Control received CCl₄ on the fourth day), and (4-6) Test Groups treated with aqueous extract at 100, 200, and 400 mg/kg for six days with CCl₄ on the fourth day). On the seventh day, blood was collected via cardiac puncture and centrifuged for liver function tests (albumin, proteins, bilirubin, ALT, AST, and ALP). Liver samples were fixed, dehydrated, paraffin-embedded, sectioned, and stained with hematoxylin-eosin for histological analysis.

3 RESULTS

3.1 PHYTOCHEMICAL SCREENING AND QUANTIFICATION OF POLYPHENOLS AND FLAVONOIDS

Polyphenols, tannins, and free quinones, known for antioxidant properties, were abundant in both extracts, while sterols and polyterpenes were absent. Saponins appeared only in the methanolic extract, highlighting methanol's effectiveness for these compounds.

Table 1. Phytochemical Screening of the Different Extracts Studied

Test	Methanolic Extract	Aqueous Extract
Polyphenols	+++	+++
Flavonoids	+	+
Tannins	+++	+++
Free Quinones	+++	+++
Sterols and Polyterpenes	-	-
Saponins	+	-

Source: Prepared by the authors (2024).

-: absence, +: presence, ++: presence in low quantity, +++: presence in abundance

The quantification of total polyphenols in various extracts of *Ceratonia siliqua* shows significant variations. The methanolic and aqueous leaf extracts have concentrations of 23.33 ± 0.45 and 43.55 ± 0.22 mg EAG/g, respectively. The flavonoid analysis in different extracts of *Ceratonia siliqua* leaves also shows notable variations. The methanolic extract has a flavonoid concentration of 3.78 ± 0.42 mg/ml, while the aqueous extract contains significantly more, with a concentration of 16 ± 0.63 mg/ml.

Table 2. Yield and Quantification of Total Polyphenols and Flavonoids in the Two Extracts Studied

	Total Polyphenols (mg EAG/g)	Flavonoids (mg/ml)	Yield (%)
EA	$43,55 \pm 0,22$	16 ± 0.63	$14,7 \pm 0.35$
EM	$23,33 \pm 0,45$	$3,78 \pm 0.42$	$26,28 \pm 0.55$

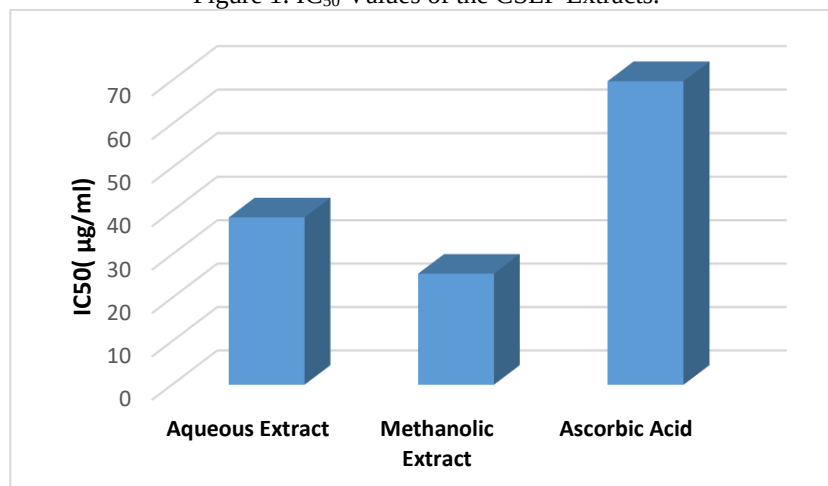
Source: Prepared by the authors (2024).

(AE): Aqueous Extract, (ME): Methanolic Extract

3.2 IN VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF CSLP EXTRACTS

The results indicate that the antioxidant properties of the samples studied were lower than those of ascorbic acid, used as a positive control (Figure 1). All tested extracts exhibited radical scavenging activity, which increased with the concentration of the extract (Figure 2). The highest antioxidant activity was observed in the methanolic extract of CSLP at a concentration of 200 µg/ml ($IC_{50} = 25.6$ µg/ml), followed by the aqueous extract of CSLP ($IC_{50} = 38.56$ µg/ml). The obtained data demonstrated that the methanolic extract has high antioxidant activity.

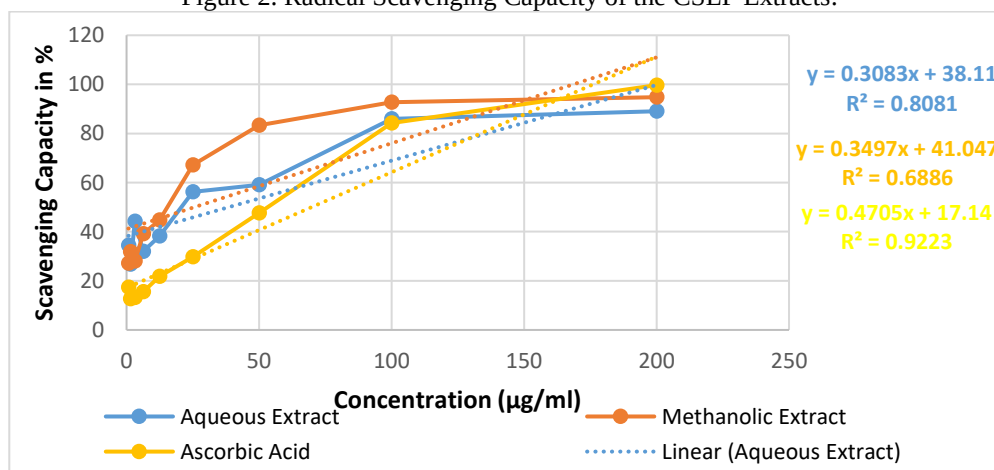
Figure 1. IC₅₀ Values of the CSLP Extracts.



Source: Prepared by the authors (2024).

All tested extracts exhibited radical scavenging activity, which increased with the concentration of the extract (Figure 2). The highest antioxidant activity was observed in the methanolic extract of CSLP at a concentration of 200 µg/ml (IC₅₀ = 25.6 µg/ml), followed by the aqueous extract of CSLP (IC₅₀ = 38.56 µg/ml). The obtained data demonstrated that the methanolic extract has high antioxidant activity. These results suggest that the methanolic extract of CSLP had a strong capacity to inhibit DPPH, due to the presence of active constituents in carob leaves capable of donating a hydrogen to a free radical, thus neutralizing the unpaired electron responsible for the radical's reactivity.

Figure 2. Radical Scavenging Capacity of the CSLP Extracts.

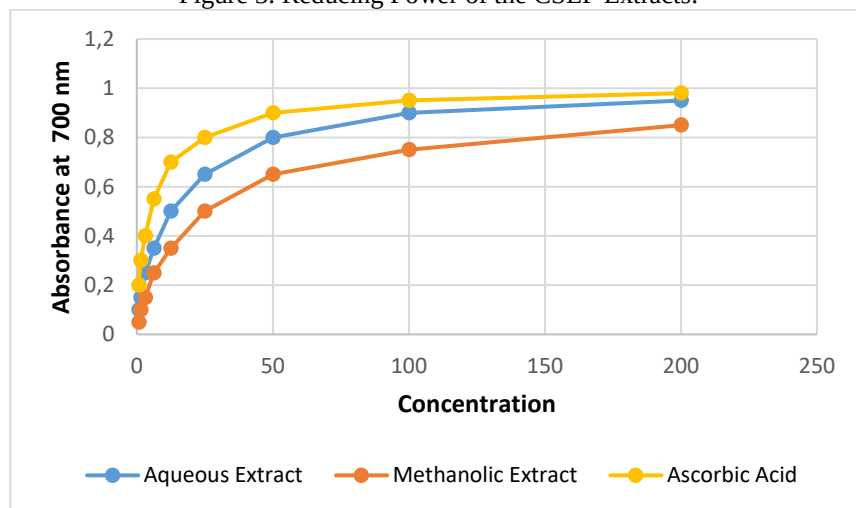


Source: Prepared by the authors (2024).

An increase in the reducing power of CSLP extracts was observed in correlation with their concentration (Figure 3). However, ascorbic acid demonstrated superior reducing power compared to CSLP extracts. Among these extracts, the methanolic extract showed higher reductive activity than the aqueous extract. The kinetics curves of β -carotene bleaching over time are represented in

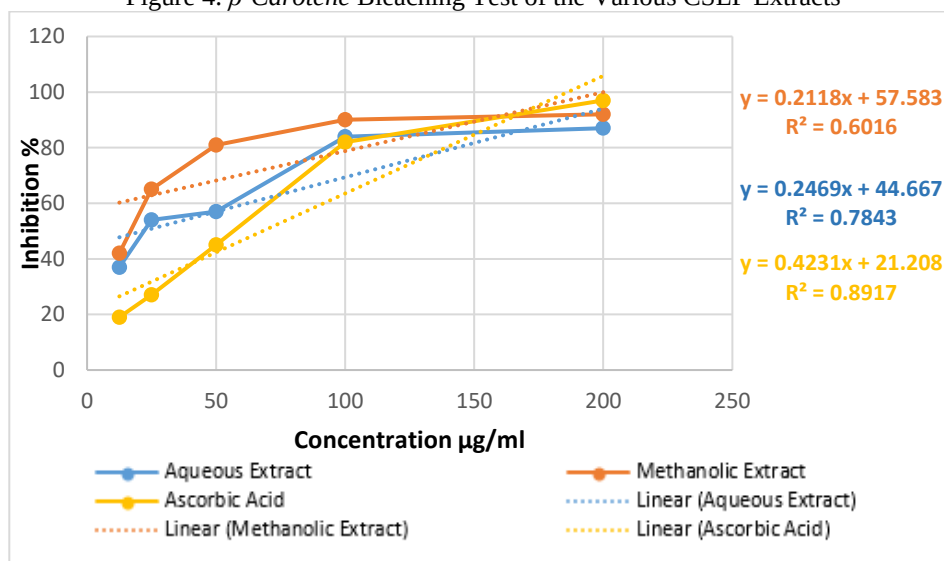
the (Figure 4). The aqueous extract demonstrated the most potent antioxidant activity with an IC₅₀ of 21.59 µg/ml, followed by the methanolic extract with an IC₅₀ of 35.8 µg/ml. Both extracts surpassed ascorbic acid (AA), used as a reference, which has an IC₅₀ of 68.05 µg/ml.

Figure 3. Reducing Power of the CSLP Extracts.



Source: Prepared by the authors (2024).

Figure 4. β -Carotene Bleaching Test of the Various CSLP Extracts



Source: Prepared by the authors (2024).

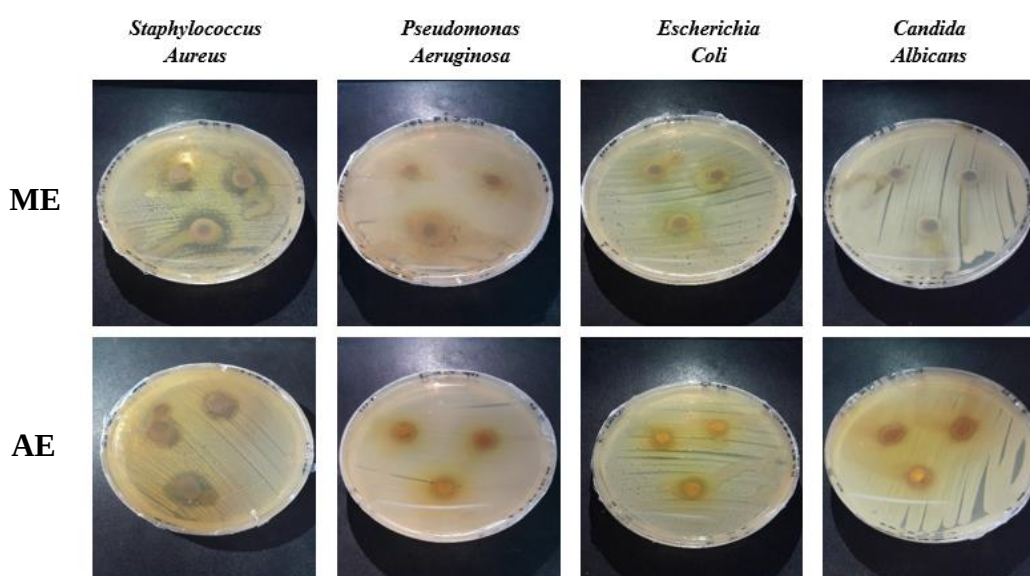
The results shown in the (Table 3) demonstrate significant antimicrobial activity of both extracts against the tested pathogens. The aqueous extract was particularly effective against *Staphylococcus aureus* with an inhibition zone of 23 mm, comparable to the positive control RD30. The methanolic extract also exhibited strong activity against *S. aureus* (22.66 mm) and superior efficacy against *Escherichia coli* (15 mm) compared to the aqueous extract. Both extracts displayed moderate activity against *Pseudomonas aeruginosa* and *Candida albicans*, with inhibition zones generally exceeding 10 mm.

Table 3. Antimicrobial Activity of CSLP Extracts

Pathogenic Microorganisms	Inhibition Zone (mm)					
	AE	ME	Positive Control			Negative Control
			ATM30	RD30	KF30	DMSO
<i>Staphylococcus aureus</i> (ATCC 25923)	23	22.66	/	40	50	/
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	10.66	11.66	17	12	/	/
<i>Escherichia coli</i> (ATCC 25922)	11	15	20	15	/	/
<i>Candida albicans</i> (ATCC 18804)	11.66	9.33	15	25	20	/

Source: Prepared by the authors (2024).
(AE): Aqueous Extract, (ME): Methanolic Extract

Figure 5. Results of Antimicrobial Activity of Aqueous and Methanolic Extracts of CSLP Against Studied Strains



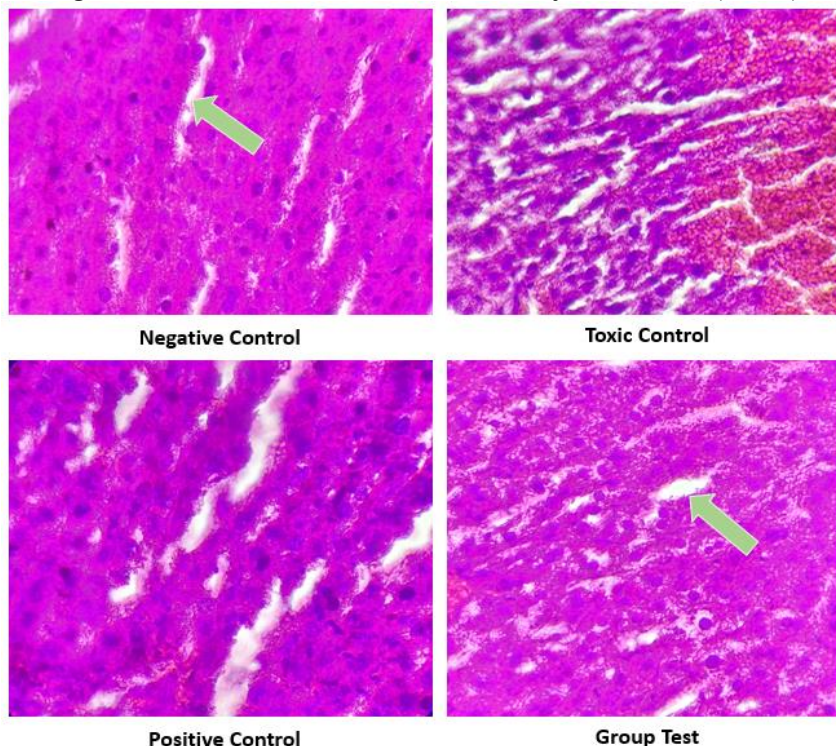
Source: Prepared by the authors (2024).

3.3 IN-VIVO HEPATOPROTECTIVE ACTIVITY STUDY

The acute oral toxicity test of the aqueous extract of CSLP did not show any significant behavioral changes or mortality during the first 24 hours and the subsequent 14 days, indicating an LD₅₀ greater than 2,000 mg/kg in mice. The hepatoprotective effect of the aqueous CSLP extract was studied in the context of liver damage induced by carbon tetrachloride (CCl₄), a well-established model of hepatic toxicity. Histopathological examinations revealed that liver sections from the negative control group exhibited normal histological architecture (Figure 6 Negative Control), as expected in the absence of toxic treatment. Mice exposed to CCl₄ without treatment (Figure 6 Toxic Control) showed significant histopathological abnormalities, such as hepatocyte disorganization, necrosis, hyperplasia, as well as infiltration and inflammation of cells. These observations corroborate the deleterious effects of CCl₄, known to cause oxidative stress and cellular damage through the formation of free radicals. In contrast, mice that received preventive or curative

treatment with the aqueous extract of *Ceratonia siliqua* at a dose of 400 mg/kg (Figure 6 Test Group) showed notable protection

Figure 6. Liver Sections Stained with Hematoxylin and Eosin (H & E).



Source: Prepared by the authors (2024).

Analysis of the hepatic profile results reveals a promising hepatoprotective effect of the aqueous CSLP extract at 400 mg/kg against CCl₄-induced damage in mice. This conclusion is supported by the significant reduction in ASAT levels (from 222.6 U/L in the toxic control group to 26.6 U/L in the test group) and ALAT levels (from 415.9 U/L to 156 U/L), which are sensitive markers of hepatocellular injury. Although less pronounced, an improvement is also observed for alkaline phosphatase (from 289 U/L to 252 U/L), total proteins (from 86.48 g/L to 71.14 g/L), albumin (from 41.26 g/L to 39.41 g/L), and total bilirubin (from 3.16 mg/L to 2.69 mg/L).

Table 4. Impact of Aqueous CSLP Extract on Biochemical Parameters in Control and Treated Mice.

	ASAT UI/L	ALAT UI/L	Alkaline Phosphatase UI/L	Total Proteins g/l	Albumin g/l	Total Bilirubin mg/l
Negative Control	4.8	13.02	168	56.62	32.8	0.78
Positive Control	11.5	131.1	176	57.83	34.49	2.54
Toxic Control	222.6	415.9	289	86.48	41.26	3.16
Test Group	26.6	156	252	71.14	39.41	2.69

Source: Prepared by the authors (2024).

4 DISCUSSIONS

The phytochemical analysis of *Ceratonia siliqua* leaves powder (CSLP) extracts revealed a rich profile of bioactive compounds, consistent with previous studies on carob leaves. Our findings align with those of (Alqudah *et al.*, 2022), who identified high levels of flavonoids, tannins, and free quinones in carob leaf extracts. Similarly, (Yahiaoui *et al.*, 2021) observed an abundance of flavonoids and tannins in both grafted and spontaneous carob varieties, with varying levels of saponins and quinones. While our methanolic and aqueous extracts contained smaller quantities of flavonoids, they demonstrated moderate extraction capability for these compounds.

The methanolic extract of CSLP proved most effective in extracting a wide range of bioactive compounds, while the aqueous extract showed moderate efficiency. Notably, our aqueous leaf extracts exhibited higher polyphenol content compared to both grafted and spontaneous varieties studied by (Yahiaoui *et al.*, 2021). These results highlight the potential of CSLP as a rich source of beneficial phytochemicals.

Our findings on flavonoid content are comparable to those reported in previous studies. (Dallali *et al.*, 2017) found flavonoid contents ranging from 0.22 to 7.42 mg EQ/g of dry matter in methanolic extracts of *Ceratonia siliqua* leaves, while another research (Vaya *et al.*, 2006) measured a flavonoid content of 2.58 mg in the ethanolic extract.

The antioxidant activity of CSLP extracts was evaluated using various assays. The methanolic extract demonstrated strong DPPH inhibition, likely due to the presence of active constituents capable of neutralizing free radicals. These results are consistent with previous studies showing carob's potent DPPH scavenging ability (Torun *et al.*, 2013; Qasem *et al.*, 2018; Ayache *et al.*, 2018). Furthermore, our findings on carob's ability to reduce Fe^{3+} to Fe^{2+} align with other studies (Abidar *et al.*, 2020) who reported strong antioxidant activity with an EC_{50} of 0.123 mg/mL in the FRAP test.

The β -carotene bleaching assay revealed that the aqueous extract outperformed the methanolic extract, suggesting that the most effective antioxidant compounds in our samples are primarily water-soluble. This observation, as described by (Elbouzidi *et al.*, 2023), highlights the promising potential of the aqueous extract as a source of natural antioxidants, with potential applications in nutrition, cosmetics, and pharmacology.

The antimicrobial activity of CSLP extracts was more pronounced than previously reported. Our results showed larger inhibition zones compared to those observed by (Kivçak *et al.*, 2002) who observed more modest inhibition zones (7-8 mm) for *E. coli*, *C. albicans*, and (7-13 mm) for *S. aureus*, suggesting potential improvements in extraction efficacy or variability in bioactive compounds depending on sample origin. The work of (Meziani *et al.*, 2015) supports our

observations, attributing the antimicrobial activity of *C. siliqua* leaf and pod extracts against *P. atrosepticum* to the presence of phenolic compounds, flavonoids, and tannins. A recent study (Al-Azzawi *et al.*, 2023) further reinforces our conclusions, demonstrating dose-dependent antimicrobial activity of the alcoholic leaf extract with significant inhibition zones for *Candida* (21 mm at 100 mg/ml), *E. coli* (19 mm), and *S. aureus* (19 mm), and also extending the known spectrum to *Bacillus cereus* and *Salmonella*. This convergence of results across different studies highlights the significant and broad-spectrum potential of CSLP extracts as a source of natural antimicrobial agents, opening promising avenues for the development of new treatments and justifying continued research into the identification and characterization of bioactive compounds responsible for these effects.

The hepatoprotective effects of the aqueous CSLP extract were evaluated in a CCl₄-induced liver damage model. The protection was evidenced by a significant reduction in inflammatory cells, vascular congestion, cellular degeneration, necrosis, and vacuolation. These results suggest that the aqueous extract exerts antioxidant and anti-inflammatory effects that mitigate CCl₄-induced liver damage. At lower doses of 100 and 200 mg/kg, the aqueous extract failed to prevent liver damage, and the mice succumbed a day after CCl₄ administration. This indicates that the hepatoprotective efficacy of the extract is dose-dependent. In (comparison, mice treated with silymarin at a dose of 100 mg/kg (Figure 6 Positive Control) showed normal hepatic structures, confirming its recognized protective role. These results are consistent with previous studies established by (Souli *et al.*, 2015), highlighting the potential of natural compounds present in *Ceratonia siliqua* pulp and seeds to offer protection against hepatic toxins. The dose-dependent efficacy of the aqueous extract of *Ceratonia siliqua* underscores the importance of determining optimal doses to maximize therapeutic benefits.

Our biochemical analysis suggests that the extract may act by stabilizing hepatic cell membranes and/or exerting antioxidant activity, thereby limiting oxidative damage caused by CCl₄. However, the protective effect appears to be partial, as the values in the test group, although improved, do not reach the levels of the negative control for most parameters. This observation underscores the need for further studies to optimize the dose, explore underlying mechanisms of action, and identify bioactive compounds responsible for this hepatoprotective effect, paving the way for potential therapeutic applications of *Ceratonia siliqua* in managing liver disorders.

This study demonstrates the significant potential of *Ceratonia siliqua* leaves powder extracts as a source of natural antioxidants and antimicrobial agents. The hepatoprotective effects observed in our animal model further highlight the therapeutic potential of these extracts. However, further studies are needed to optimize dosage, explore underlying mechanisms of action, and identify specific bioactive compounds responsible for these effects. This research paves the way for potential

therapeutic applications of *Ceratonia siliqua* in managing liver disorders and other health conditions.

5 CONCLUSION

The foliar material of *Ceratonia siliqua* L. shows significant potential as a reservoir of bioactive compounds. Chemical analysis revealed high polyphenol content in the aqueous extract (43.55 mg EAG/g). Both extracts exhibited strong antioxidant activity, with methanolic extract IC_{50} of 25.6 $\mu\text{g/ml}$ (DPPH assay) and aqueous extract IC_{50} of 21.59 $\mu\text{g/ml}$ (β -carotene test). Significant antimicrobial activity, particularly against *Staphylococcus aureus*, and hepatoprotective effects at 400 mg/kg against CCl_4 -induced liver injury were observed. These results emphasize its pharmaceutical and therapeutic potential, warranting further research.

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