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Ephedra alata Extracts as Promising Biotherapeutics Against Infection and Inflammation

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Abstract

Ephedra alata (Ephedraceae) is a medicinal plant traditionally used in North African and Middle Eastern folk medicine to treat respiratory infections, inflammation, and pain. This study aimed to evaluate the antibacterial and anti-inflammatory activities of aqueous and ethanolic extracts from *E. alata* aerial parts collected in Oued Al Alenda, Algeria, to provide pharmacological evidence supporting its traditional uses.

Aqueous and ethanolic extracts were obtained by hot maceration and ethanol soaking, respectively. Phytochemical screening revealed the presence of polyphenols, flavonoids, and alkaloids, with saponins detected only in the aqueous extract. Antibacterial activity was assessed by the disk diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (2–5 mg/mL). Anti-inflammatory activity was evaluated using a carrageenan-induced paw edema model in rats, with Diclofenac as reference.

The ethanolic extract showed a higher yield (20.30%) than the aqueous extract (15.09%) and exhibited strong antibacterial effects against *S. aureus* (17 mm) and *P. aeruginosa* (15 mm). The aqueous extract was moderately active against *P. aeruginosa* (14.5 mm) and *E. coli* (13.5 mm). In vivo, the ethanolic extract inhibited paw edema by 54% after 4 h, comparable to Diclofenac (50%), while the aqueous extract achieved 33%. C-reactive protein levels were significantly reduced by both extracts.

These findings confirm the strong antibacterial and anti-inflammatory potential of *Ephedra alata*, particularly its ethanolic extract, supporting its relevance as a natural biotherapeutic source for infection and inflammation management.

Keywords: *Ephedra alata*, antibacterial activity, anti-inflammatory effects, ethanolic extract, aqueous extract.

1. Introduction

The abundance of bioactive substances in medicinal plants has made them an attractive alternative for research; the growing demand for natural products in the food, cosmetic, and pharmaceutical industries is a sign of this growing interest (Davis & Choisy, 2024; Laib & Djahra, 2022; Wong-Paz et al., 2015). Among the countries rich in plant biodiversity, Algeria stands out with its 3164 species of medicinal plants, widely used by traditional healers for their therapeutic properties (Benarba et al., 2021a).

Ephedra alata belongs to the genus *Ephedra*, with the Arabic common name "Alanda." The plant is native to several Middle Eastern countries, including Saudi Arabia, Egypt, Iran, Algeria, Lebanon, Jordan, Iraq, Libya, Tunisia, and Morocco (Jaradat et al., 2021). The plant stands out among these plants due to its many medicinal qualities, especially its antioxidant , antibacterial and anti-inflammatory capabilities (Bahri et al., 2022; Jaradat et al., 2021; Mahmoudi et al., 2023).

According to Bahri et al., (2022) and Abourashed et al. (2003), this plant has long been used to treat a variety of illnesses, including allergies, bronchial asthma, colds, fevers, and congestion of the nose, However, it has also been used to treat bacterial and fungal infections, cancer, and abnormalities of the digestive system (Bahri et al., 2022). Its ability to combat bacterial infections is demonstrated by its efficacy against pathogens that cause infectious disorders, including Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus (Al-Snafi, 2017).

Phytochemical analyses of *Ephedra alata* have revealed that it is a valuable source of secondary metabolites, known for their numerous biological activities. Exploring its antibacterial properties is a dynamic and promising field of research (González-Juárez et al., 2020).Numerous phytochemicals, including kaempferol, quercetin, resveratrol, p-coumaric acid, and caffeic acid, have been detected in substantial concentrations in the aerial portions of the plant (Danciu et al., 2018; Nimse & Pal, 2015).

However, alkaloids including ephedrine, pseudoephedrine, norephedrine, and methylephedrine, as well as tannins and flavonoids, are linked to the medical and therapeutic actions of these plants. Ephedrine and pseudoephedrine are the most prevalent substances in Ephedra (Dousari et al., 2022).

Recent studies have highlighted its antioxidant and anti-inflammatory properties, while research in Tunisia has focused on its remarkable anticancer properties, particularly against breast cancer cells (Al Jaafreh, 2024; Benarba et al., 2021b; Mohammed et al., 2024; Saidi et al., 2022). These studies underscore the vast bioactive potential of *Ephedra alata*, which could combat infections and treat inflammatory , diabetic and cancerous diseases.

The present study aims to systematically investigate the antibacterial and anti-inflammatory properties of aqueous and ethanolic extracts of *Ephedra alata*, a medicinal plant traditionally used in North African folk medicine. The research aims to assess its efficacy against clinically relevant bacterial strains, including *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*, using disc diffusion assays to determine its spectrum of antimicrobial activity. Additionally, the anti-inflammatory effects are evaluated through both biochemical (CRP

levels) and cellular markers (neutrophil, monocyte, and platelet counts), as well as in vivo models of carrageenan-induced paw edema in rats. By correlating phytochemical composition with biological activity, the study seeks to elucidate the extract-specific mechanisms of action and determine which solvent system yields the most therapeutically potent formulation. Ultimately, the goal is to provide scientific validation for the traditional use of *Ephedra alata* and to promote its potential as a safe, effective, and accessible source of natural anti-infective and anti-inflammatory agents for integration into both local and global healthcare strategies.

2. Materials and methods

2.1. Collection region of plant

The plant material comes from *Ephedra alata*, collected in February 2024 in the municipality of Oued Al Alenda (33°01'43"N 6°47'02"E), located west of El-Oued, Algeria. This Saharan region is characterized by a hyper-arid climate, where summers are particularly hot and dry, with temperatures reaching 45°C (Houmri et al., 2023; Khezzani & Bouchemal, 2017).

2.2.Bacterial Strains Used

The bacterial strains used in this study were reference strains obtained from the American Type Culture Collection (ATCC), including *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 8737). The strains were maintained at 5 °C in sterile tubes containing 10 ml of nutrient agar medium to preserve viability prior to testing.

2.3.Preparation of the Aqueous Extract

The aqueous extract was prepared by hot maceration of 40 g dried plant powder in 200 ml of water at 100 °C, followed by 24 hours of magnetic stirring. The solution was filtered, evaporated at 50 °C for 30 minutes, then dried in a 45 °C oven for 3 days.

2.4.Preparation of the Ethanol Extract

The ethanol extract was obtained by soaking 40 g of plant powder in 200 ml of 70% ethanol with continuous stirring for 72 hours. After filtration, the residue was re-macerated using the same method. Both filtrates were combined, evaporated at 50 °C, and dried in open crystallizers at 45 °C for 3 days.

2.5. Evaluation of the yield of extracts

The yield percentage of the dried extracts was calculated using the following equation (Adnan et al., 2010):

$$Yield\% = \frac{(W_1 \times 100)}{W_2} \qquad (1).$$

where W_1 represents the weight of the extract after solvent evaporation, and W_2 denotes the initial weight of the *Ephedra alata.*

2.6. Phytochemical Screening

The aqueous extract of *Ephedra alata* was subjected to qualitative tests to identify various phytochemical constituents. Alkaloids were confirmed through Mayer's and Wagner's reagents, which produced cream-colored and brownish/reddish-brown precipitates,

respectively (Abdo et al., 2015). Polyphenols were detected by the appearance of colored complexes upon the addition of 5% ferric chloride solution (Llorent-Martínez et al., 2023). Flavonoids were identified through the reaction with dilute ammonia and concentrated sulfuric acid, resulting in a yellow coloration that gradually faded over time (Llorent-Martínez et al., 2023). Lastly, saponins were detected by their ability to generate stable foam when the extract was vigorously shaken with distilled water (Alqethami & Aldhebiani, 2021).

2.7. Quantification of Total polyphenol

Total polyphenol content was determined using the method of Singleton and Rossi (1965), based on the colorimetric reaction between polyphenols and the Folin–Ciocalteu reagent. In this procedure, 0.2 ml of crude extract at varying concentrations was mixed with 1 ml of 10% Folin–Ciocalteu reagent and 0.8 ml of sodium carbonate (Na_2CO_3). After thorough mixing, the reaction mixture was allowed to stand for 30 minutes at room temperature. Absorbance was then measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid was used as the reference standard for the calibration curve. All measurements were performed in triplicate.

2.8. Quantification of Flavonoids

Flavonoid content was determined according to the method described by Alia (2010), using aluminum chloride ($AlCl_3$) complexation. Briefly, 750 µl of crude extract at various concentrations was mixed with 750 µl of a 2% $AlCl_3$ solution. The mixture was thoroughly vortexed and incubated at room temperature for 1 hour. Absorbance was then measured at 420 nm using a UV-Vis spectrophotometer.

2.9. Antibacterial activity

The antibacterial activity of Ephedra alata aqueous and ethanolic extracts was evaluated using the disk diffusion method, following a previously established protocol. Bacterial strains were preserved at 5°C in sterile nutrient agar slants to maintain viability. To initiate the assay, 20 ml of sterilized Mueller-Hinton agar was poured into sterile Petri dishes and allowed to solidify under aseptic conditions. Pure bacterial colonies were isolated, suspended in physiological saline, and adjusted to a 0.5 McFarland standard, ensuring consistent bacterial density. The suspension was evenly spread onto Mueller-Hinton agar plates using sterile cotton swabs to achieve a uniform bacterial lawn.

Sterile Whatman paper disks (6 mm in diameter) were impregnated with 10 µl of Ephedra alata extracts at varying concentrations (5, 4, 3, and 2 mg/ml) and carefully placed on the inoculated agar surface. A disk saturated with DMSO served as a negative control to confirm the absence of solvent-induced inhibition, while standard antibiotic discs (Amoxicillin, Co-trimoxazole, and Ciprofloxacin) were used as positive controls for comparative analysis.

The plates were incubated at 37°C for 24 hours to promote bacterial growth and assess the extracts' inhibitory potential. Antibacterial efficacy was determined by measuring the diameters of inhibition zones surrounding the disks, with larger zones indicating greater antimicrobial potency (LAIB et al.).

2.10. Evaluation of the anti-inflammatory activity (acute inflammation) of the aqueous and ethanolic extract of *Ephedra alata* in rats

To assess the anti-inflammatory activity of the aqueous and ethanolic extracts of *Ephedra alata*, an acute paw edema model induced by 1% carrageenan was used in rats. Sodium diclofenac served as the reference anti-inflammatory drug.

2.10.1. Anti-inflammatory activity

Paw edema was induced in rats by subcutaneous injection of 1% carrageenan, following the method described by Winter et al. (1962)**.** Male rats weighing between 170 g and 200 g were fasted for 24 hours prior to the experiment. The animals were randomly divided into four groups of five rats each. The diameter of the right hind paw was measured at baseline (T_0) using a digital caliper, according to the procedure outlined by Bukhari (2013) .

The experimental groups were arranged as follows:

• **Group 1 (Edema control):** Received distilled water.

• **Group 2 (Reference group):** Treated with Diclofenac Sodium at a dose of 20 mg/kg body weight.

• **Group 3:** Treated with the aqueous extract at a dose of 500 mg/kg body weight.

• **Group 4:** Treated with the ethanolic extract at a dose of 500 mg/kg body weight.

One hour after oral administration of the test solutions, 0.1 ml of 1% carrageenan was injected subcutaneously into the plantar aponeurosis of the left hind paw of each rat. The development of paw edema was then monitored at 1, 2, 3, and 4 hours post-injection.(Suralkar et al., 2012), and at 24h. The extent of the edema was assessed by calculating the average percentage increase (%AUG) in paw volume using the following formula:

$$UG\% = \frac{(V_n - V_0)}{V_0} \times 100 \qquad (2)$$

$\boldsymbol{V_n}$ **:** Paw volume at time t.

$\boldsymbol{V_0}$: Initial paw volume.

The anti-inflammatory activity was also assessed by determining the percentage of edema inhibition (%INH) using the following formula:

$$INH\% = \frac{(\boldsymbol{AUG\%\ Injecte - AUG\%\ traitées})}{\boldsymbol{AUG\%\ Injecte}} \times 100 \qquad (\mathbf{3})$$

2.11. Blood sampling and analysis of inflammatory markers

Blood samples were collected from the tail into EDTA tubes for hematological analysis and into dry tubes for immunobiochemical analysis, specifically for the measurement of C-Reactive Protein (CRP). Samples were taken at three time points: before product administration (baseline), 4 hours after administration, and 24 hours post-administration..

2.12. Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical differences were evaluated using one-way ANOVA. A p-value less than 0.05 was considered statistically significant. Analyses were performed using SPSS software (version 26).

3. Results and discussion

3.1. Yields and distribution of bioactive compounds in aqueous and ethanol of Ephedra alata

The extraction yield of Ephedra alata varied significantly between the two solvents used, with the ethanolic extract yielding 20.30%, compared to 15.098% for the aqueous extract **(Table 1).** This difference reflects the polarity and solubility of bioactive compounds present in the plant material. Ethanol, being a semi-polar solvent, is capable of extracting a broader spectrum of phytochemicals, including both polar and moderately non-polar constituents such as flavonoids, alkaloids, phenolics, and terpenoids (Dbeibia et al., 2024). In contrast, water, a highly polar solvent, is more selective for hydrophilic compounds such as tannins, saponins, and certain glycosides (Markom et al., 2007). The higher yield obtained with ethanol suggests that Ephedra alata contains a substantial proportion of compounds that are more soluble in ethanol than in water (Nagarajan et al., 2016). This result is consistent with previous studies on other medicinal plants where ethanolic extracts often exhibit superior extraction efficiency and biological activity due to their ability to solubilize diverse secondary metabolites (Laib et al., 2024). These findings underscore the significance of solvent selection in phytochemical research, suggesting that ethanol may be a more effective solvent for extracting pharmacologically relevant constituents from Ephedra alata, thereby potentially enhancing its antibacterial and anti-inflammatory properties (Mondol et al., 2013; Zheng et al., 2021).

Table 1. Yield of Aqueous and Ethanol Extracts of *Ephedra alata*

Extracts	**Yield (%)**
Aqueous Extracts	15.098 (%)
Ethanol Extracts	20.30 (%)

3.2. Qualitative and quantitative analysis of aqueous and ethanol of *Ephedra alata extact*

The phytochemical screening of Ephedra alata extracts revealed the presence of key bioactive compounds in both aqueous and ethanolic extracts, with some notable differences. Qualitatively, both extracts tested positive for polyphenols, flavonoids, and alkaloids (Wagner's test), while they were negative for alkaloids using Mayer's reagent **(Table 2)**. Interestingly, saponins were detected only in the aqueous extract, indicating that these compounds are more soluble in polar solvents, such as water, consistent with their hydrophilic nature (Edrah et al., 2016; Tlili et al., 2025) . The presence of polyphenols and flavonoids in both extracts aligns with the traditional medicinal use of Ephedra alata and supports its potential for exhibiting antioxidant, antibacterial, and anti-inflammatory activities (Jaradat et al., 2015; Segueni et al., 2025).

Quantitatively, the aqueous extract contained a higher total polyphenol content (TPC) at 0.712 ± 0.324 mg GAE/g extract, compared to 0.537 ± 0.209 mg GAE/g in the ethanolic extract. Conversely, the total flavonoid content (TFC) was significantly greater in the ethanolic extract (0.188 ± 0.209 mg QE/g) than in the aqueous extract (0.063 ± 0.324 mg QE/g). These results suggest that while water is more efficient in extracting polyphenolic compounds, ethanol is

more effective in solubilizing flavonoids, which are typically less polar (Bouafia et al., 2025; Cuevas-Valenzuela et al., 2014). This variation in phytochemical composition may influence the biological activity of each extract, as polyphenols and flavonoids are both implicated in antimicrobial and anti-inflammatory mechanisms through pathways involving free radical scavenging, inhibition of pro-inflammatory mediators, and disruption of microbial membranes (Mello et al., 2010).

Overall, the phytochemical profiles highlight the complementary nature of aqueous and ethanolic extracts in capturing a broad spectrum of bioactive molecules from Ephedra alata, reinforcing the relevance of solvent choice in optimizing therapeutic efficacy (Irakli et al., 2018; Mello & Hubinger, 2012; Tlili et al., 2024).

Table 2. Results of the phytochemical screening and quantitative analysis of the *Ephedra alata* aqueous extract.

Qualitative analysis		
Phytochemical Compounds	**Aqueous Extract**	**Ethanol Extracts**
Polyphenols	(+)	(+)
Alkaloids (Mayer)	(-)	(-)
Alkaloids (Wagner)	(+)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(-)
Quantitative analysis		
Phytochemical Compounds	**Aqueous Extract**	**Ethanol Extracts**
TPC (mg GAE/g extract)	0.712± 0.324	0.537±0.209
TFC (mg QE /g extract	0.063±0.324	0.188±0.209

3.3. Antimicrobial activity

The antibacterial activity of *Ephedra alata* extracts demonstrated a concentration-dependent inhibitory effect, with varying degrees of effectiveness across the three bacterial strains tested: *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The aqueous extract exhibited pronounced antibacterial activity particularly against *E. coli* and *P. aeruginosa*, with inhibition zones increasing from 8 mm at 3 mg/mL to 13.5 mm and 14.5 mm respectively at the highest concentration of 5 mg/mL **(Table 3)**. Notably, no inhibition was observed against *S. aureus* at any concentration of the aqueous extract, suggesting the limited efficacy of polar phytochemicals against this Gram-positive bacterium.

In contrast, the ethanolic extract showed strong antibacterial activity primarily against *S. aureus* and *P. aeruginosa*. The most significant inhibition was observed at 5 mg/mL, with zones of 17 mm and 15 mm, respectively. However, the ethanolic extract exhibited minimal activity against

E. coli, with a modest inhibition of 7.5 mm only at the highest tested concentration. This differential activity may reflect the selective solubility of bioactive compounds in ethanol that are more effective against Gram-positive organisms like *S. aureus*, which have a thicker peptidoglycan layer but lack the outer membrane barrier present in Gram-negative bacteria.

When compared to standard antibiotics, ciprofloxacin, amoxicillin, and co-trimoxazole all demonstrated significantly higher antibacterial potency with inhibition zones exceeding 22 mm across all bacterial strains. Ciprofloxacin was the most effective, especially against *E. coli* (41 mm), indicating the superior activity of synthetic antibiotics. Nonetheless, the moderate zones of inhibition recorded for *Ephedra alata* extracts at higher concentrations suggest that the plant possesses bioactive compounds with genuine antibacterial potential, albeit less potent than conventional antibiotics.

These findings highlight the broad-spectrum activity of *Ephedra alata*, especially in combating *Pseudomonas aeruginosa*, a notoriously resistant pathogen (Mufti et al., 2023). The observed antibacterial effects are likely due to the presence of flavonoids and polyphenols identified in the phytochemical screening, known to exert membrane-disruptive and enzyme-inhibiting actions (Dbeibia et al., 2023; Rajhard et al., 2021). The complementary activity profiles of aqueous and ethanolic extracts underscore the value of solvent polarity in extracting compounds targeting different bacterial types (Ni et al., 2019). This supports the potential use of *Ephedra alata* as a source of natural antimicrobial agents, particularly in regions where antibiotic resistance is a growing concern (Mufti et al., 2023).

Table 3. Mean diameters of inhibition zones for *Ephedra alata* extracts and antibiotics against different bacterial strains tested.

Sample /concentrations		***Escherichia coli***		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
Aqueous extracts	**2mg/ml**	/	-	/	-	8mm	+
	3mg/ml	8mm	+	/	-	9mm	+
	4mg/ml	10mm	+	/	-	11.5mm	+
	5mg/ml	13.5mm	+	/	-	14.5mm	+
Ethanolic extracts	**2mg/ml**	/	-	9mm	+	/	-
	3mg/ml	/	-	9.5mm	+	/	-
	4mg/ml	/	-	10mm	+	/	-
	5mg/ml	7.5mm	-	17mm	+	15 mm	+
Antibiotic	**Ciprofloxacin (CIP)**	41 mm	+++	28.5 mm	+++	22.5 mm	+++
	Amoxicillin (AX)	24.5 mm	+++	33.5 mm	+++	52.5 mm	+++
	Co-Trimoxazole (COT)	31 mm	+++	27 mm	+++	15 mm	++

3.4. Anti-inflammatory activity

3.4. 1. Evaluation of the effects of different products on edema induced by carrageenan

Figure 1 illustrates the evolution of paw edema volume in rats over a 24-hour period following carrageenan-induced inflammation and subsequent treatment with either *Ephedra alata* extracts (aqueous and ethanolic) or diclofenac, a standard anti-inflammatory drug. The carrageenan-only group (red bars) exhibited a progressive increase in paw volume, peaking at 3–4 hours post-injection, confirming the successful induction of acute inflammation, consistent with the well-characterized biphasic response of the carrageenan model.

Remarkably, both *Ephedra alata* extracts demonstrated substantial anti-inflammatory effects, with varying degrees of efficacy (Silva et al., 2010). The aqueous extract (green bars) significantly reduced paw edema compared to the untreated inflammatory group, particularly at the 3rd and 4th hour, indicating potent activity during the late phase of inflammation, which is typically mediated by prostaglandins and other cytokines (Koliyote & Misal, 2013). Interestingly, the ethanolic extract (turquoise bars) also reduced inflammation but showed a slightly more pronounced effect during the early to mid-phase (1h to 3h), suggesting the presence of active compounds that may interfere with early mediators such as histamine and serotonin (Sadeghi et al., 2011).

Diclofenac (pink bars), as expected, exhibited the highest anti-inflammatory efficacy throughout the time course, maintaining consistently low paw volumes. However, it is noteworthy that both *Ephedra alata* extracts approached the activity of diclofenac, particularly the aqueous extract at the 4h and 24h marks, highlighting their potential as natural alternatives or complementary agents in anti-inflammatory therapy.

The observed anti-inflammatory activity can be attributed to the presence of bioactive constituents such as flavonoids, polyphenols, and alkaloids (Wagner positive), known for their antioxidant and enzyme-inhibiting properties that suppress inflammatory mediators (Alsuwayt, 2025; Ibrahim et al., 2024). The aqueous extract's superior efficacy at later stages may be linked to its higher total phenolic content, which contributes to sustained anti-inflammatory action (Makni et al., 2019).

These results strongly support the traditional use of *Ephedra alata* in folk medicine for treating inflammatory conditions (Ahmed et al., 2025). They also underscore the potential of both extracts, especially the aqueous one, as promising candidates for developing plant-based anti-inflammatory agents with fewer side effects compared to synthetic drugs (Najmi et al., 2022).

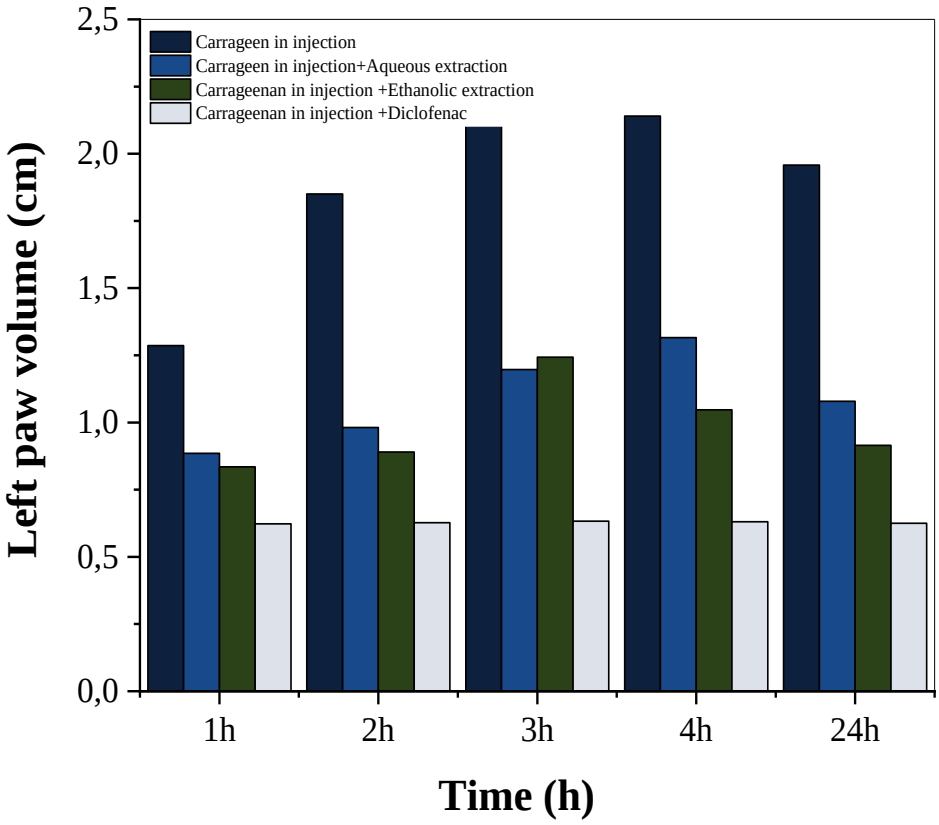


Figure 1. Volume of the left paw in rats after carrageenan injection and treatment with *Ephedra alata* extracts and Diclofenac (mean±SD).

Figure 2 demonstrates the inhibitory effect of *Ephedra alata* aqueous and ethanolic extracts, compared to the standard drug diclofenac, on carrageenan-induced paw edema in rats over a 24-hour period. The ethanolic extract consistently exhibited superior anti-inflammatory activity compared to the aqueous extract and displayed inhibition levels closely matching those of diclofenac, particularly during the peak inflammatory phases. At 1 hour post-carrageenan injection, the ethanolic extract showed 41% inhibition, higher than both diclofenac (37%) and the aqueous extract (28%), suggesting a rapid onset of action, possibly through suppression of early mediators such as histamine and serotonin. The anti-inflammatory activity intensified at 2–4 hours, aligning with the secondary phase of inflammation driven by prostaglandins and other pro-inflammatory cytokines. Notably, at the 4-hour mark, the ethanolic extract achieved 54% inhibition—virtually equivalent to diclofenac (50%)—while the aqueous extract reached 33%, confirming that the ethanolic extract is more potent.

This strong performance of the ethanolic extract is likely attributed to its higher solubility of lipophilic bioactive compounds such as flavonoids, alkaloids, and tannins, which are known to inhibit cyclooxygenase and lipoxygenase pathways, reduce oxidative stress, and downregulate

inflammatory cytokines like TNF-α and IL-6 (Plaskova & Mlcek, 2023; Uttra et al., 2018). The aqueous extract, although less effective, still demonstrated significant anti-edematous activity, possibly due to the presence of hydrophilic phenolics and polysaccharides that contribute to membrane stabilization and reduced vascular permeability (Yaseen et al., 2020). At 24 hours, the persistence of inhibitory activity—45% for the ethanolic and 31% for the aqueous extract—indicates a prolonged therapeutic effect, which is desirable for chronic inflammatory conditions (Khattabi et al., 2022; Mufti et al., 2023).

Overall, the results validate the traditional use of *Ephedra alata* in treating inflammatory disorders and highlight the ethanolic extract as a particularly promising candidate for natural anti-inflammatory drug development. Its performance, comparable to that of diclofenac, supports the idea that phytochemicals within *Ephedra alata* can offer a multi-targeted approach with fewer side effects than synthetic drugs. These findings justify further phytochemical characterization and mechanistic studies to isolate the active compounds and elucidate their pathways of action in inflammation control.

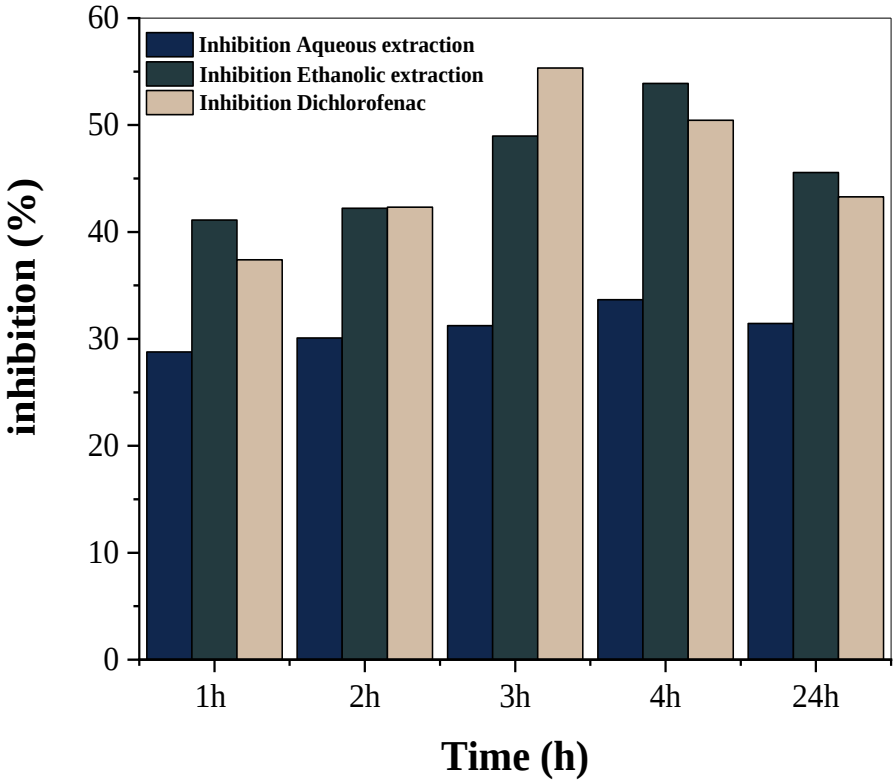


Figure 2. Inhibitory effect on edema of *Ephedra alata* extracts and Diclofenac.

3.5. Anti-inflammatory effect on the expression of neutrophil , monocyte and platelets

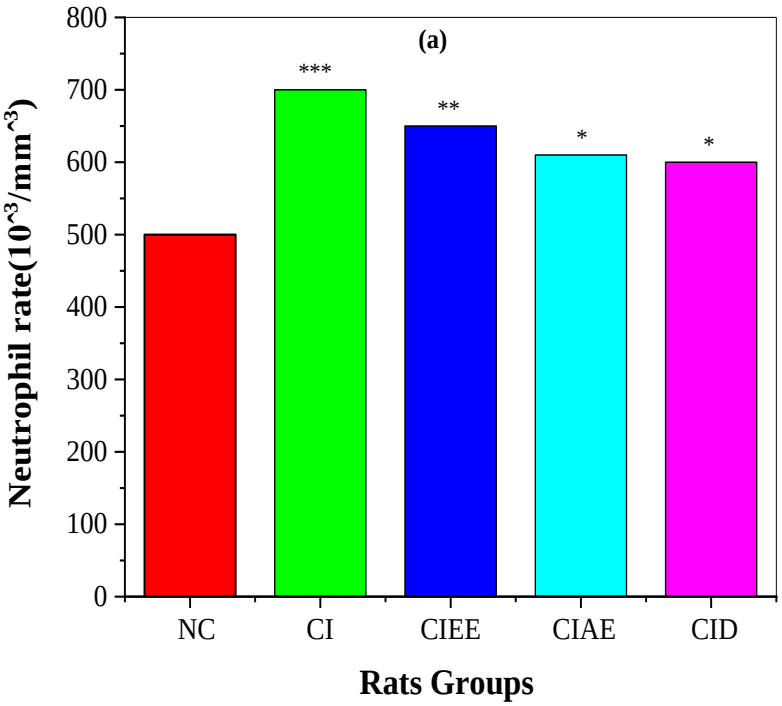
The carrageenan-induced inflammation model elicited a pronounced immunohematological response, characterized by significant increases in neutrophil, monocyte, and platelet counts, confirming the development of acute inflammatory processes. Specifically, neutrophil levels

rose from 500 $\times 10^3/mm^3$ in the control group (Tem) to 720 $\times 10^3/mm^3$ in the carrageenan-only group (Car), reflecting strong innate immune activation and chemotactic response **(Figure 3).** Simultaneously, monocyte counts increased from 0.9 to 1.1 $\times 10^3/mm^3$, indicating enhanced antigen-presenting cell mobilization, while platelet numbers escalated from 570 to 700 $\times 10^3/mm^3$, suggesting amplified thrombo-inflammatory interactions commonly associated with tissue injury and edema formation.

Remarkably, co-treatment with *Ephedra alata* extracts demonstrated a robust ability to attenuate these inflammatory surges. The ethanolic extract emerged as particularly effective, reducing neutrophils to 610 $\times 10^3/mm^3$, monocytes to 1.0 $\times 10^3/mm^3$, and platelets to 630 $\times 10^3/mm^3$. These reductions are nearly comparable to those achieved by Diclofenac (neutrophils: 600 $\times 10^3/mm^3$, monocytes: 0.98 $\times 10^3/mm^3$, platelets: 590 $\times 10^3/mm^3$), a standard non-steroidal anti-inflammatory drug. The aqueous extract also yielded significant improvements, lowering neutrophil levels to 640 $\times 10^3/mm^3$, monocytes to 0.995 $\times 10^3/mm^3$, and platelets to 615 $\times 10^3/mm^3$, indicating a consistent anti-inflammatory profile, albeit slightly less potent than its ethanolic counterpart.

The mechanism behind this activity can be attributed to the rich phytochemical composition of *Ephedra alata*, particularly its flavonoids, alkaloids, tannins, and saponins, which have been widely reported to exert antioxidant, membrane-stabilizing, prostaglandin-inhibiting, and cytokine-modulating effects (Linke et al., 2017; Mufti et al., 2023). These bioactives likely inhibit the release of pro-inflammatory mediators such as TNF-α, IL-1β, and COX-2, while simultaneously impairing leukocyte migration and platelet aggregation (Zhang et al., 2019).

In light of these results, it becomes clear that *Ephedra alata*, especially in its ethanolic form, displays strong immunomodulatory and anti-inflammatory properties, rivaling the efficacy of synthetic drugs (Mufti et al., 2023). Its dual action in controlling both leukocyte infiltration and thrombotic potential positions it as a promising candidate for managing acute and possibly chronic inflammatory conditions (Zamora et al., 2021). These findings not only validate its ethnomedicinal use but also open new avenues for the development of safer, plant-based anti-inflammatory therapies (Banik et al., 2020).


(a)
Neutrophil rate(10^3/mm^3)
800
700
600
500
400
300
200
100
0
***
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*
*
NC
CI
CIEE
CIAE
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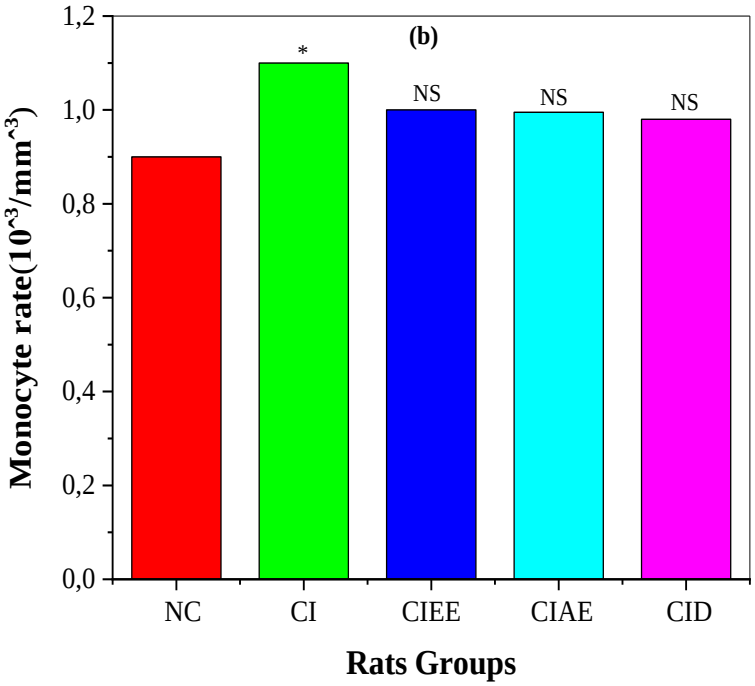
Rats Groups


(b)
Monocyte rate(10^3/mm^3)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
*
NS
NS
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CI
CIEE
CIAE
CID
Rats Groups

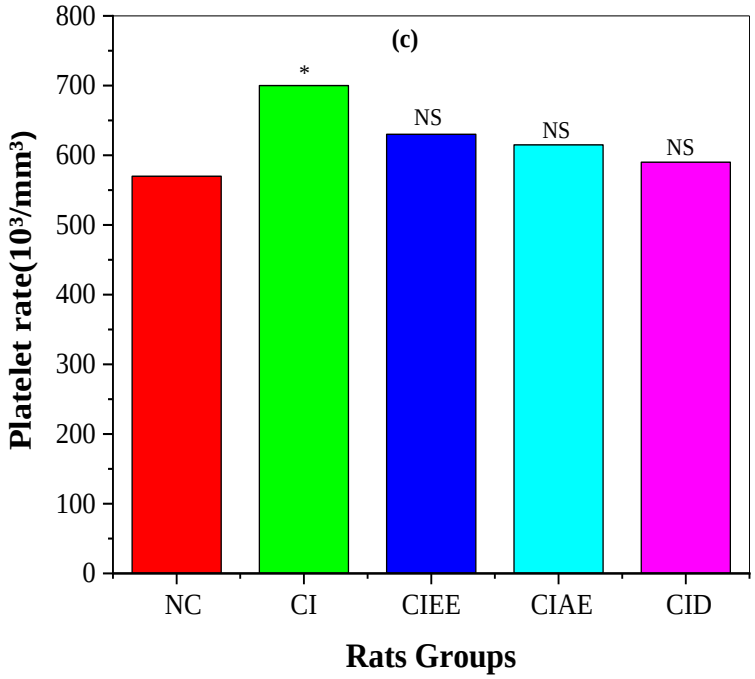

Figure 3. Modulatory Effects of *Ephedra alata* Extracts and Diclofenac on Neutrophil (a), Monocyte (b), and Platelet (c) Counts in Carrageenan-Induced Inflammatory Rats. Data are presented as mean ± SD (n = 20). Statistical significance: NS ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). NC: Tem, CI: Carrageenan-Induced Inflammation, CIEE: Carrageenan + Ephedra alata Ethanolic Extract, CIAE: Carrageenan + Ephedra alata Aqueous Extract, CID: Carrageenan + Diclofenac.

3.6 . Evaluation of serum CRP levels after edema induction by carrageenan

C-Reactive Protein (CRP) is a critical acute-phase biomarker that serves as a sensitive systemic indicator of inflammation, produced predominantly by hepatocytes in response to pro-inflammatory cytokines such as interleukin-6 (IL-6), TNF-α, and IL-1β **(Figure 4).** In this study, the baseline CRP level in healthy control rats (Tem) was 0.998 mg/L, reflecting a state of normal physiological homeostasis devoid of any significant inflammatory challenge.

However, after carrageenan injection, which induces robust acute inflammation, the CRP levels skyrocketed to 89 mg/L, underscoring the successful establishment of systemic inflammation. This massive elevation confirms the inflammatory cascade induced by carrageenan, characterized by vascular leakage, cellular infiltration, and enhanced hepatic CRP synthesis (Ou et al., 2019). The high CRP value is consistent with the activation of nuclear factor kappa B (NF-κB) and the systemic dissemination of pro-inflammatory mediators (Wu et al., 2020).

Importantly, therapeutic intervention with *Ephedra alata* extracts demonstrated substantial anti-inflammatory efficacy, as evidenced by a marked reduction in CRP levels (Mufti et al., 2023; Thadani, 2018). Treatment with the ethanolic extract resulted in a significant decline of CRP to 56 mg/L, indicating an approximate 37% reduction compared to the carrageenan-only

group. Even more impressively, the aqueous extract lowered CRP levels further to 38 mg/L, achieving a ~57% reduction, nearly matching the effect of Diclofenac, which brought CRP down to 34 mg/L—a 61.7% reduction.

These findings suggest that both aqueous and ethanolic extracts of *Ephedra alata* possess notable systemic anti-inflammatory activity, with the aqueous extract surprisingly exhibiting slightly greater CRP-lowering potential than the ethanolic extract (Chávez et al., 2025). This discrepancy may be attributed to the different polarities of the solvents used, which extract diverse sets of bioactive compounds (Cao et al., 2018). The aqueous extract might be richer in water-soluble phenolics, flavonoids, and saponins, all known to inhibit COX enzymes, block cytokine release, and suppress hepatocyte-mediated CRP synthesis (Mukhopadhyay et al., 2023). Meanwhile, the ethanolic extract, though potent, may contain more lipophilic alkaloids and terpenoids with complementary but slightly less systemic efficacy in this model.

Comparing these natural extracts to Diclofenac, a widely used NSAID that operates via COX inhibition and prostaglandin suppression, highlights the remarkable therapeutic promise of *Ephedra alata*. While Diclofenac remains a gold standard, its potential for gastrointestinal, renal, and cardiovascular side effects calls for safer alternatives. In contrast, *Ephedra alata* offers a multifactorial anti-inflammatory mechanism, potentially involving antioxidant defense enhancement, modulation of cytokine pathways, and stabilization of lysosomal membranes, all contributing to reduced systemic CRP production (Tsoupras et al., 2024).

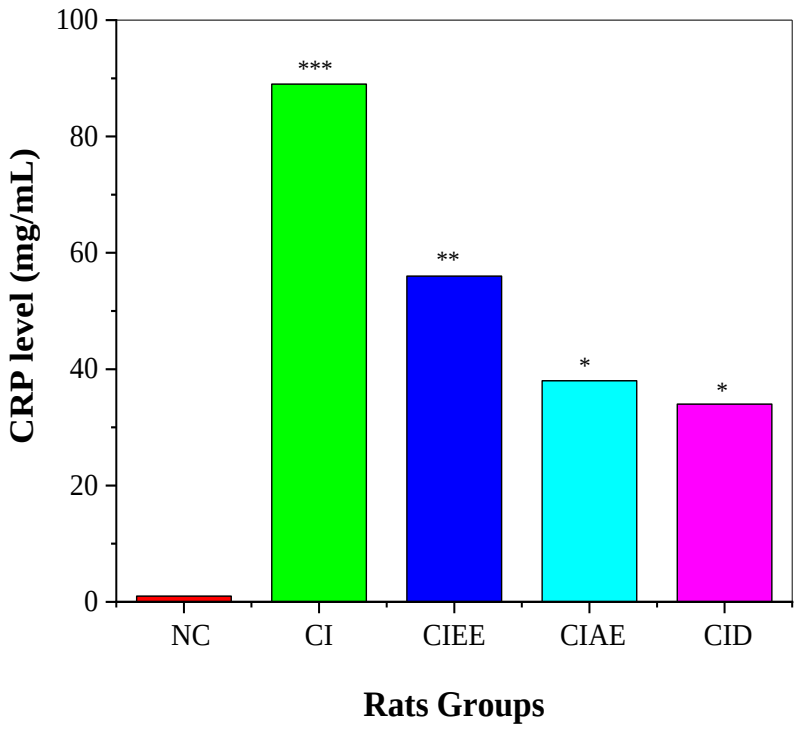

Figure 4. C-Reactive Protein (CRP) levels in the treated rat groups. Data are expressed as mean ± SD. Significance indicated as : * :p < 0.05, ** : < 0.01 , *** : p < 0.001, n = 20. NC: Tem,

CI: Carrageenan-Induced Inflammation, CIEE: Carrageenan + Ephedra alata Ethanolic Extract, CIAE: Carrageenan + Ephedra alata Aqueous Extract, CID: Carrageenan + Diclofenac.

4. Conclusion

The thorough study of *Ephedra alata* has revealed a remarkable richness in bioactive metabolites, confirming its potential as a promising source of natural treatments. On the one hand, phytochemical and biological analyses have demonstrated that this plant possesses significant antibacterial activity against several pathogenic strains, including resistant bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These results reinforce the idea that aqueous and ethanolic extracts of *Ephedra alata* contain compounds capable of inhibiting pathogens responsible for severe human infections. On the other hand, studies conducted on the anti-inflammatory activity of this plant have revealed remarkable effects. The ethanolic extract has shown efficacy comparable to that of Diclofenac, a reference anti-inflammatory drug, suggesting the presence of mechanisms similar to those of nonsteroidal anti-inflammatory drugs (NSAIDs). This anti-inflammatory activity has been confirmed by the modulation of immune cells, such as neutrophils and monocytes, without disrupting hematological balance. This is a major advantage compared to conventional treatments.

Thus, the results of this study confirm that *Ephedra alata* represents a promising natural alternative not only for treating bacterial infections but also for managing acute inflammation, with reduced risks of side effects. These discoveries pave the way for the broader use of this plant in the pharmaceutical field and place *Ephedra alata* at the forefront of new natural therapeutic strategy development.

Statements and Declarations

Ethics Approval and Consent to Participate: All animals were sourced from the Pasteur Institute, Algeria, with animal certification number 30 CMB/130/2020. The study adhered to ethical standards set by the Institutional Animal Ethics Committee (IAEC) and the NIH Guide for the Care and Use of Laboratory Animals. Twenty adult male albino rats (170–200 g) were acclimatized for two weeks at the Department of Molecular and Cellular Biology, University of El-Oued, Algeria. They were housed under a 12-hour light/dark cycle at 19 ± 1 °C, with access to standard rat chow and tap water ad libitum.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Competing interests: The authors declare no conflict of interest. Financial or otherwise.

Authors' Contributions: Conceptualization, N.H., B.K., G.R., S.G., M.A., I.L., and A.B.; methodology, N.H., B.K., G.R., S.G., M.A., I.L., and A.B.; validation, N.H., B.K., G.R., and A.B.; investigation, S.G., M.A., I.L., and A.B.; resources, N.H., B.K., and A.B.; data curation, G.R., S.G., M.A., I.L., and A.B.; writing—original draft preparation, N.H., B.K., G.R., S.G., M.A., I.L., and A.B.; writing—review and editing, N.H., B.K., G.R., S.G., M.A., I.L., and A.B.; supervision, B.K., G.R., A.B.; All authors have read and agreed to the published version of the manuscript.

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