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Research Article

Phytochemical Composition and Biological Activities of *Ephedra alata* Decne.: Antioxidant, Anti-Inflammatory and Anticancer Potentials

Bachir Benarba[a,b*], Khadidja Belhouala[a,c], Cansu Korkmaz[d], Meltem Taş Küçükaydın[e], Selçuk Küçükaydın[f] and Mehmet Emin Duru[e]

[a]Laboratory Research on Biological Systems and Geomatics, Mustapha Stambouli University of Mascara, Mascara, 29001, Algeria
[b]Thematic Research Agency in Health and Life Sciences, Oran, Algeria
[c]Univesity of Ain Temouchent, Ain Temouchent, 46000, Algeria
[d]Department of Biology, Faculty of Science, Muğla Sıtkı Koçman University, 48000, Muğla, Turkey
[e]Department of Chemistry, Faculty of Science, Muğla Sıtkı Koçman University, 48000 Muğla, Turkey
[f]Department of Medical Services and Techniques, Köyceğiz Vocational School of Health Services, Muğla Sıtkı Koçman University, 48800, Köyceğiz-Muğla, Turkey
*Corresponding author: b.benarba@atrss.dz

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ABSTRACT

Ephedra alata Decne. is a medicinal species used to treat several ailments including cancer, digestive and respiratory disorders, bacterial and fungal infections, and renal failure. Although its therapeutic uses are documented, comprehensive *in vitro* evaluation of its anti-inflammatory, antioxidant, and anticancer potential using a phytochemical-based approach has remained limited. This study focused on the evaluation of anticancer (MTT assay), anti-inflammatory (HRBC membrane stabilizing, proteins denaturation and protease inhibition assays), and antioxidant activities (TAC, DPPH, reducing power, and H_2O_2), in addition to the phenolic characterization of *E. alata* extracts. The phytochemical compounds were identified by HPLC-DAD analysis. Additionally, the anti-inflammatory, antioxidant and anticancer potential were assessed in vitro. Rutin, vanillin, p-hydroxy benzoic acid, pyrocatechol, luteolin, and quercetin were the phenolic compounds revealed in the aerial part of *E. alata* by HPLC-DAD. The methanolic and aqueous extracts exhibited an important radical scavenging activity with IC_{50} value of 535 ± 0.035 and 644 ± 0.048 µg/mL, respectively. Impressively, the methanolic extract was particularly effective in reducing hypotonic-induced HRBC lysis with 42.80% protection (IC_{50} value of 12.22 ± 0.04 µg/mL). Unfortunately, no potent anticancer effects were exerted against the carcinoma cells. However, the extracts from *E. alata* were found not to be toxic to normal cells. Taken together, these findings suggest that the phytochemicals of *E. alata* contributed to the biological activities and could be considered as natural sources of antioxidant and anti-inflammatory agents, although no considerable anticancer activity was observed here.

Keywords: *Ephedra alata*, anticancer, antioxidant; anti-inflammatory, HPLC-DAD

1. INTRODUCTION

Plants are considered promising sources of active molecules owing to their phytochemical diversity (Olalekan, 2023; Shahidi et al., 2015). Their compounds have interesting biological properties, acting mainly as antioxidant, antimicrobial, anti-inflammatory, and antitumor agents (Ramirez et al., 2024; Salleh and Khamis, 2020; Ozcan et al., 2014; Carocho and Ferreira, 2013). Of these plants, some common species revealed their effects in experimental studies by identifying their bioactive constituents and measuring their biological activities to ensure their pharmacological benefits. Others were reported only in ethnobotanical research, as they are traditionally used by local people in many countries to eliminate several diseases (Belhouala and Benarba, 2021; Salleh and Ahmad, 2016). In Algeria, folk medicine took an important place in the Algerian culture, exhibiting numerous ethnobotanical approaches with interesting flora. This "continent-country" is characterized by unique biodiversity due to the geographical and climatic diversity in each region. Numerous species such as *Artemisia herba-alba, Bryonia dioica*, and *Aristolochia longa* have been shown to contain promising bioactive chemicals with significant biological potential, including antimicrobial, anticancer, and anti-inflammatory (Ziani et al., 2018; Benarba, 2016).

Ephedra alata Decne., a Saharan species, is a small and perennial stiff shrub, about 50-100 cm tall of the genus *Ephedra* (Al-Rimawi et al., 2017). This medicinal plant is widely distributed across arid environments, often near shifting sand dunes, and grows on gravely rocky, sandy, and clay soils. It is native to Algeria, Iraq, Iran, Chad, Egypt, Palestine, Lebanon, Jordan, Saudi Arabia, Morocco, Syria, Libya, Mauritania, Mali, Somalia, and Tunisia (Jaradat et al., 2015). *E. alata* is a perennial genus of non-flowering seeds with light green densely branched dioecious with therapeutic properties. The stem of *E. alata* has long been used in traditional medicine for the treatment of kidney, bronchi, circular system, and digestive system disorders, as well as relief from asthma attacks and bacterial and fungal infections. Furthermore, the aerial parts of this plant have been attributed to cancer treatments. Indeed, it has been reported that *E. alata* presents in vitro anticancer activities, inhibiting proliferation and inducing apoptosis, which suppress tumor angiogenesis (Shukla and Mehta, 2015). In spite of some previous studies reporting cytotoxic activities, systematic or comprehensive in vitro investigations assessing the antioxidant, anti-inflammatory and cytotoxic effects of the plant are lacking. On the other hand, the phenolic profile of *E. alata*, especially those growing in the Algerian Sahara, remains poorly characterized using advanced techniques such as HPLC-DAD. Therefore, the present study aims to fill these gaps by providing new insights into the therapeutic potential of this underexplored Saharan medicinal plant and supports its rational use in natural product drug discovery.

Here, the main objectives were to evaluate the antioxidant and anti-inflammatory potential and emphasize the anticancer properties against three human cancer cells (HT-29, PC-3, A549), in addition to the phytochemical investigation of *E. alata* extracts.

2. MATERIALS AND METHODS

2.1. Plant Material

Fresh aerial parts of *E. alata* (Voucher No.007.LRSBG/AB/21/12) were gathered on June 6, 2021 from Adrar, Sahara region in southwestern Algeria (Figure 1). The sample was cleaned, dried, and crushed using a grinder (Laboratory IKA M20 universal grinding mill) to recover the powder of *E. alata* aerial parts and then stored in a black container. The botanical identification was performed at the Department of Biology (University of Mascara, Algeria).

Figure 1. Shoot system of *E. alata*

2.2. Chemicals and Reagents

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), methanol, ethanol, hydrochloric acid, glacial acetic acid, ferric chloride, concentrated sulphuric acid, magnesium ribbon, Hager's reagent, sodium hydroxide solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphomolybdate reagent, phosphate buffer, and 30% H_2O_2 solution were obtained from Sigma Aldrich.

2.3. Preparation of the Plant Extracts

Aqueous extract: A total of 20 g of dried aerial part powder was mixed with 200 mL of distilled water heated to 100°C with continuous stirring for 20 min, cooled to room temperature, and then filtered using a filter paper (WhatmanNo.42). The residue was re-extracted twice following the same process. All the filtrates obtained were combined, concentrated, lyophilized, and stored at 4°C until further analysis (Yadav and Agarwala, 2011).

Methanolic extract: To prepare the methanol extract, 40 g of the dried aerial part was macerated for seven days with 400 mL of methanol, and the mixture was constantly stirred at room temperature and stored overnight at 4°C. (Sioud et al., 2020). The resulting extracts were filtered, concentrated at 40°C with reduced pressure in a rotary evaporator, and then dried. Before being used, the crude extracts were collected and kept at 4°C.

2.4. Phytochemical Screening

The phytochemical screening of *E. alata* aerial part extracts was conducted qualitatively and quantitatively using standard color and precipitation methods as previously described (Trease and Evans, 2002; Sofowora, 1993; Marsoul et al., 2020; Alqethami & Aldhebiani, 2021). The total phenolic content (TPC) and total flavonoid content (TFC) were determined as previously described by Hosu et al. (2014), expressed as mg gallic acid equivalents (mg GAE/g extract) and mg quercetin equivalents (mg QE/g extract), respectively. The total tannin content (TTC) was analyzed using the vanillin assay (Bikoro et al., 2018) and expressed as mg catechin equivalents (mg CE/g of extract). Under standard conditions, the phenolic profile analysis of *E. alata* was carried out by the HPLC-DAD technique as described by Deveci et al. (2019); and Çayan et al. (2020). The phenolic compounds were monitored at 280 nm with a photodiode array detector (PDA) and then identified in terms of retention times and UV data by comparison with commercial standards. The calibration curve was obtained by injecting the standard compounds at concentrations between 0.0 and 1.0 ppm. The analyses were repeated three times

and the results were provided as mg/g extract dry weight (dw) (Deveci et al., 2019; Çayan et al., 2020; Tel-Çayan et al., 2022).

2.5. Antioxidant Activity

The total antioxidant capacity (TAC) was evaluated using the phosphomolybdate assay (Ahmed et al., 2014). The results were expressed as mg/g of ascorbic acid equivalents (mg/g AAE). The DPPH radical scavenging activity of *the E. alata* extracts was estimated according to the method of Brand-Williams et al. (1995) and was expressed as percent inhibition. The reducing power was determined using the method of Oyaizu et al. (1986), with the absorbance measured at 700 nm. The hydrogen peroxide (H_2O_2) scavenging activity was evaluated following the method described by Ruch et al. (1989), with the absorbance measured at 230 nm. The results were expressed as the percentage scavenging activity.

2.6. Anti-inflammatory Activity

2.6.1. HRBC Membrane Stabilizing Assay

The human red blood cell (HRBC) membrane stabilization activity of the plant was evaluated via heat and hypotonic solution-induced hemolysis as described by Azeem et al (2010). HRBCs were prepared from O+ human blood (donors provided written informed consent in accordance with the Declaration of Helsinki) and suspended in PBS. Results were expressed as percentage of protection.

2.6.2. Effect on Protein Denaturation

The inhibition of heat-induced protein denaturation was performed using bovine serum albumin and egg albumin according to the two methods described by Sunmathi et al. (2016). The inhibition percentages were calculated based on the absorbance readings at 255 and 660 nm, respectively.

2.6.3. Protease Inhibition Assay

The protease inhibitory activity of the *E. alata* extracts was evaluated as previously described by Oyedepo et al. (1995) using trypsin and casein. After reading the absorbance at 210 nm, the results were expressed as the inhibitory percentage.

2.7. Anticancer Activity

Cells were seeded in 96-well plates at a concentration of 1×104 cells/well for 24 h in a humidified environment with 5% CO_2 and 95% air. The cells were then treated with increasing concentrations of *E. alata* extracts for 72 h. After adding 10 mL of MTT (0.5 mg/mL), incubation for 4 h and removing the media, 100 µL of pure DMSO was added to dissolve the formazan blue crystals that had formed, and the absorbance was then measured at 540 nm on a microplate reader. All samples were analyzed in triplicate, and the anticancer potential was calculated as the cell viability percentage.

2.8. Statistical Analysis

With GraphPad Prism version 8 software, the data were presented as the mean ± standard deviation (SD) of three independent experiments. They were then subjected to one-way analysis of variance (ANOVA) and Tukey's multiple comparison, with a significance level of $\rho<0.05$.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield

According to the results (Table 1), methanol produced the highest extraction yield (17.83%), followed by the aqueous extract (14.50%). The extraction yields in this case were directly influenced by the polarity of the solvent, the solute's solubility in the solvent, and the technique used (Cowan, 1999). Furthermore, because maceration is often carried out at room temperature, the time needed for the methanolic extraction process is longer than that of the decoction method, which may account for the variation in the extraction yields (Ngamwonglumlert et al., 2017).

Table 1. Extraction yields, total phenolic, flavonoid and tannin contents of *E. alata* extracts

Extracts	Yield%	Phenols mg AGE/g	Flavonoids mg QE/g	Tannins mg CE/g
Aqueous	14.50 ± 0.09****	137.50 ± 0.03****	63.19 ± 0.17****	63.72 ± 0.12****
Methanolic	17.83 ± 0.05	145.83 ± 0.55	85.51 ± 0.00	75.94 ± 0.07

****<0.0001

3.2. Phytochemical Screening

The qualitative screening of *E. alata* was performed by applying coloring tests to both extracts obtained from decoction and maceration in water and methanol, respectively. The results revealed the presence of diverse secondary metabolites: cyanins, quinones, glycosides, saponins, steroids, phytosteroids, flavonoids, phenols, terpenoids, reducing sugars, alkaloids, and cardiac glycosides in both extracts. Besides, coumarins were only detected in the methanolic extract, whereas anthraquinones were not detected. In agreement with our findings, a study by Bousenna et al. (2022) showed the presence of phenols, reducing sugars, flavonoids and tannins, alkaloids, triterpenoïdes and steroids in the aerial part of Algerian *E. alata* (Bousenna et al., 2022). Similarly, *E. alata* extract represented a source of polyphenolic compounds such as flavones, flavanols, bisflavanols, carboxylic acids and alkaloids (Abourashed et al., 2003). Moreover, the methanolic extract exhibited a high presence of phenolic acids, tannins, flavonoids, cardiac glycoside, alkaloids, reducing sugars and saponins (Jaradat et al., 2015).

Regarding the quantitative phytochemical composition, we found that the TPC was significantly higher in the *E. alata* methanolic extract (972.50 ± 0.001 mg GAE/g) compared with the aqueous extract (650.83 ± 0.001 mg GAE/g) ($p \leq 0.01$). Conversely, the TFC of the aqueous extract (582.57 ± 0.013 mg QE/g) significantly exceeded that of the methanolic extract (364.58 ± 0.009 mg QE/g) by almost 60% ($p \leq 0.05$). In the same line, previous research reported a variable concentration of phenols (214.92 mg AGE/g extract) and flavonoids (30.74 mg QE/g extract) in the methanolic extract from the aerial parts of *E. alata* (Bousenna et al., 2022). In another study, *E. alata* fruits showed the presence of a higher amount of total flavonoids (98.95 mg RUE/g extract) (Jaradat et al. 2021). Moreover, the TPC and TFC were found to be 125.73 mg AGE/g and 27.89 mg QE/g extract, respectively, in the aerial parts (Danciu et al., 2018; Dbeibia et al., 2021). Additionally, a recent investigation reported a low amount of phenol and flavonoid content in ethyl acetate extract (46.66 ± 3.064 mg GAE/g extract 23.3 ± 0.504 mg QE/g extract) and butanol extract 19.62 ± 0.244 mg GAE/g extract; 10.39 ± 0.193 mg QE/g extract obtained from *E. alata* growing in Ouargla, South Algeria (Chebouat et al., 2023). Interestingly, all these findings were observed to be lower than ours, which could be related to several factors influencing the quality and quantity of phytochemicals in the plant, of them the

dissimilarity of geographical location and time of harvest, selected part and the interaction between solvent, extraction method and plants.

3.3. Characterization of Phenolic Compounds

Regarding the HPLC-DAD analysis, the phenolic compounds and organic acid composition of *E. alata* samples are listed in Table 2. Although twenty-six phenolic compound and organic acid standards were tested, only six standard compounds namely pyrocatechol, *p*-hydroxy benzoic acid, Vanillin, Rutin, Quercetin, and Luteolin were revealed in the *E. alata* extracts. Here, the main compound detected in methanolic extract was rutin (29.40 mg/g) followed by vanillin (12.52 mg/g) while this last was mainly detected to be the major compound in the aqueous extract. Contrary to methanolic extract, rutin was only detected in the aqueous extract with an amount of 1.54 mg/g. The remained compounds were found in small quantities in both samples of *E. alata* aerial part ranging from 1.54 to 9.21 mg/g. A similar investigation confirmed the presence of rutin in the *E. alata* aerial part extract in addition to other compounds such as gallic acid, catechin, caffeic acid, ferulic acid, and quercetin (Soumaya et al., 2020). In contrast with our findings, kaempferol (28.68 µg/mL), resveratrol 10.387 (µg/mL) and quercetin 4.57 (µg/mL) were mainly detected in the hydroalcoholic extracts of *E. alata* aerial part in other analyses conducted via LC-MS. However, rutin was not detected in the ethanolic extract in this previous investigation (Danciu et al., 2018). Furthermore, a study by Mahmoudi et al (2023) reported low amounts of gallic acid, quercetin, cirsiliol, naringin, and epicatechin in the extract of *E. alata* seeds (Mahmoudi et al., 2023). On the other hand, isoflavones and flavonol derivatives were found to be the major compounds in *E. alata* extracts (Ziani et al. 2019). Additionally, Khattabi et al. (2022) characterized 21 compounds in the aerial parts of *E. alata*, including caffeic acid, gallic acid, quercetin-O-rhamnoside, hyperoside, quercetin-3-O-galactoside, isorhamnetin-3-O-glucoside, quercetin-3-O-glucoside, and epicatechin (Khattabi et al., 2022).

Table 2. Phenolic composition of *E. alata* roots extracts by HPLC-DAD (mg/g extract)

No	Phenolic compounds	RT (min)	EA-Met	EA-Aqu	Class
1	Pyrocatechol	11.04	7.68	1.90****	Benzenediols
2	p-hydroxy benzoic acid	12.77	9.21	4.75****	Phenolic acids
3	Vanillin	17.78	12.52	13.02	Phenolic acids
4	Rutin	25.30	29.40	1.54****	Flavonoids
5	Quercetin	30.43	2.97	3.43	Flavonoids
6	Luteolin	31.70	3.05	2.87	Flavonoids

****<0.0001

3.4. Antioxidant Activity

As shown in Figure 2(A), at a concentration of 1 mg/mL, the total antioxidant capacity of the methanolic extract from *E. alata* aerial part reached 7.23 ± 0.04 mg/g AAE, which was slightly higher that that of the aqueous extract (7.18 ± 0.03 mg/g AAE), although no significant statistical difference was found ($p \geq 0.05$). Consistent with our results, Soumaya et al. (2020) reported that *E. alata* ethanol extract exhibited the highest antioxidant activity (280 mg GAE/g DW) compared to ethyl acetate extract of other species namely *E. fragilis* (131 mg GAE/g DW) in which the antioxidant activity was influenced by the solvent type affected (Soumaya et al., 2020).

As Figure 2(B) illustrates, a dose-dependent increase in the DPPH radical scavenging activity was recorded for both extracts. At the highest concentration of 500 ug/mL, the scavenging activity of the aqueous extract of the aerial part of *E. alata* reached 48.98%, while

at the same concentration, that of the methanolic was 47.10% with IC_{50} values of 535 ± 0.035 and 644 ± 0.048 µg/mL ($p \leq 0.05$), respectively. Moreover, the DPPH radical scavenging abilities of both extracts of *E. alata* were moderately less than that of the standard (49.86%). The ethanol extract of *E. alata* possessed a high content of polyphenolic compounds and significant antioxidant effects (DPPH: IC_{50} value 3.37 ± 0.1 µg/mL) (Soumaya et al. 2020). Furthermore, Chebouat et al. (2023) showed an effective to quench the stable-colored DPPH with IC_{50} values of 0.228 and 0.581 mg/mL for ethyl acetate and butanol extracts obtained from *E. alata* growing in Ouargla region (Southeast Algeria), respectively (Chebouat et al., 2023). Another research performed on Tunisian *E. alata* reported that the seeds extract possessed a potential antioxidant in DPPH with IC_{50} value of 14.9 µg/mL (Mahmoudi et al., 2023).

As illustrated in Figure 2(C), the methanolic extract of *E. alata* exhibited a significantly higher reducing power of 2.15 mg AAE/g at the concentration of 500 µg/mL compared with the aqueous extract (1.70 mg AAE/g, $p \leq 0.05$). In agreement with our findings, the aerial parts of *E. alata* ethanolic extract showed higher reducing power than the aqueous extract with an EC_{50} value of 262 µg/mL (Soumaya et al., 2020). The same effect was found in ethanolic extract of Palestinian *E. alata* (Jaradat et al., 2015). Nevertheless, Ziani et al. (2019) proved that hydroethanolic extracts had lower reducing power than that of aqueous extracts (infusions and decoctions) with EC_{50} values of 377 ± 4.10, 108 ± 1 µg/mL, respectively (Ziani et al., 2019).

The H_2O_2 radical scavenging activity of the *E. alata* extracts is illustrated in Figure 2(D). The scavenging activity of the aqueous extract was markedly increased from 29% to 57% in a dose-dependent manner ($p \leq 0.01$). Likewise, the methanolic extract showed a scavenging ability of 58% ($p \leq 0.05$), which slightly exceeded the scavenging capacity of ascorbic acid under the same conditions ($p < 0.05$). These data corroborate that *E. alata* possesses important scavenging effects against hydroxyl radical (Kose and Dogan, 1995).

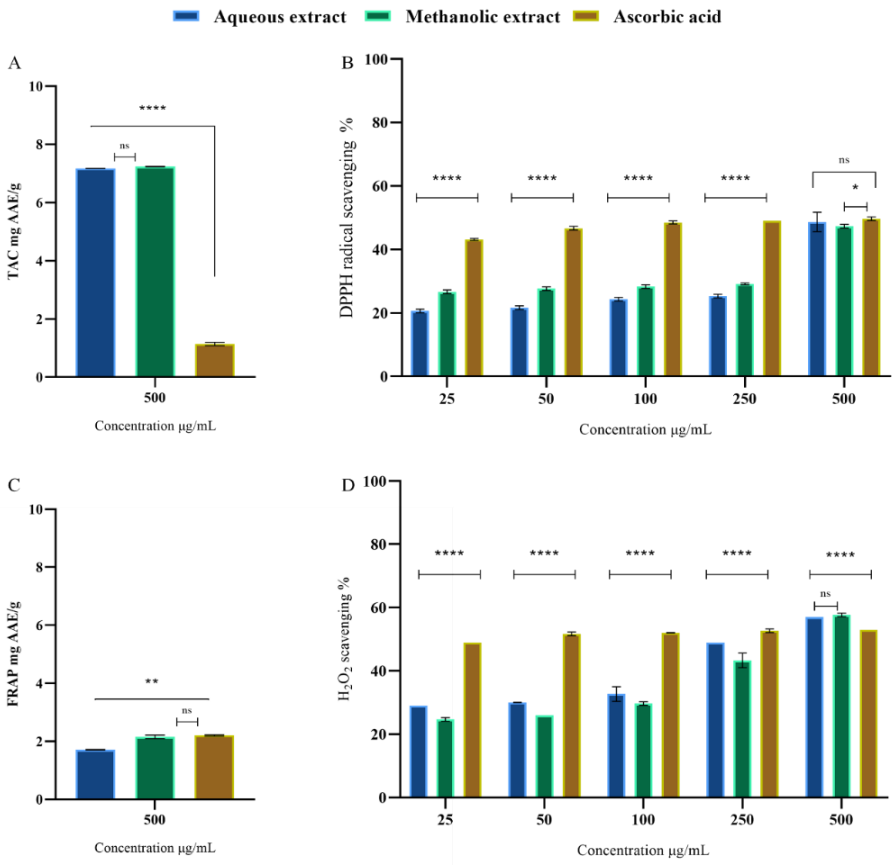

Figure 2. Antioxidant activity of *E. alata* aqueous and methanolic extracts [**(ρ<0.0001);**** (ρ<0.0001)]

Overall, the antioxidant activity was found to be correlated to the phenolic content. In fact, in several herbal products, numerous studies reported that the antioxidant activity was positively related to the total phenolic content (Zohdi et al., 2025; Mohd et al., 2023; Oktay et al., 2003). On the other hand, despite the antioxidant potential exhibited by both extracts, the methanolic extract was more effective regarding the reducing power, whereas the aqueous extract possessed better radical scavenging activity, possibly due to the differences in the chemical profiles of both extracts.

3.5. Anti-inflammatory Activity

Figure 3 showed that the aerial part of *E. alata* can be considered as a strong hemolysis inhibitor contributing to its anti-inflammatory effects. Indeed, the methanolic extract exhibited significantly higher HRBC membrane stabilization activity compared to the aqueous extract and the standard drug. At 500 µg/mL, the methanolic extract resulted in the maximum inhibitory activity of 42.80 ± 0.12%, which was significantly higher than that of the aqueous extract (29.34 ± 0.09%, p≤0.01) and that of the anti-inflammatory drug (19.73 ± 0.89%, p≤0.001). Chouikh (2020) reported the erythrocytes protective activity of *E. alata* extract, in which at a concentration of 1 mg/mL the hemolysis percentage was reduced from 55.46 to 22.27%.

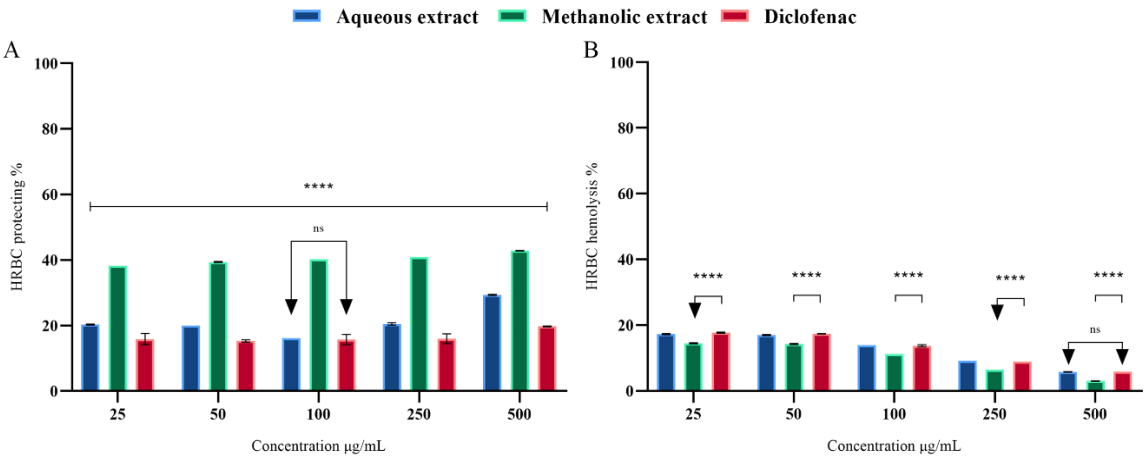

Figure 3. HRBC membrane stabilization activity (**A**) and hemolysis percentage (**B**) of *E. alata* extracts compared to s-DCF [**** (ρ<0.0001)]

As shown in Figure 4, both extracts exhibited a dose-dependent inhibition of bovine serum albumin (BSA) and egg albumin denaturation (EA). At 500µg/mL, the aqueous extract resulted in a slightly higher inhibition of BSA denaturation (57.46 ± 0.05%) than the methanolic extract (54.52 ± 1.77%, p≥0.05). Similarly, both aqueous and methanolic extracts inhibited egg albumin denaturation by 31.48 ± 0.28 and 31.95 ± 0.27%, respectively (p≥0.05). Nonetheless, both extracts showed lower inhibition when compared to the standard diclofenac sodium (96.09 ± 0.82% and 75.60 ± 0.31%, p<0.001) at the same concentration. In our previous study, we reported a notable inhibition of serum albumin denaturation (99.22 ± 0.02% and 89.74 ± 0.60%, p<0.001) at the dose of 50 µg/mL and egg albumin denaturation (73.31 ± 0.90% and 71.66%, p<0.001) at the dose of (2000 µg/mL), induced by aqueous and methanolic extracts, respectively. The effect of both extracts was higher than the standard diclofenac sodium (Benarba et al., 2021). Here, the difference in the protection action percentage between aqueous and methanolic extracts could be related to the dissimilarity in the concentration of phenolic compounds such as vanillin (13.02 and 12.52 mg/g extract, respectively). Vanillin is considered

as anti-inflammatory agent with important effect to protect against liver injury and fibrosis. Indeed, vanillin was found to protect against CCl4-induced acute liver injury through reduction of the levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and prevented CCl4-induced hepatic cell alteration and liver necrosis (Makni et al., 2011). Likewise, Eun-Ju et al. (2008) demonstrated the anti-inflammatory activity of vanillin that inhibited the production of nitric oxide (NO), induction of inducible nitric oxide synthase (iNOS) and reduced the iNOS mRNA level in the LPS-activated macrophages in ICR Mice (Eun-Ju et al., 2008).

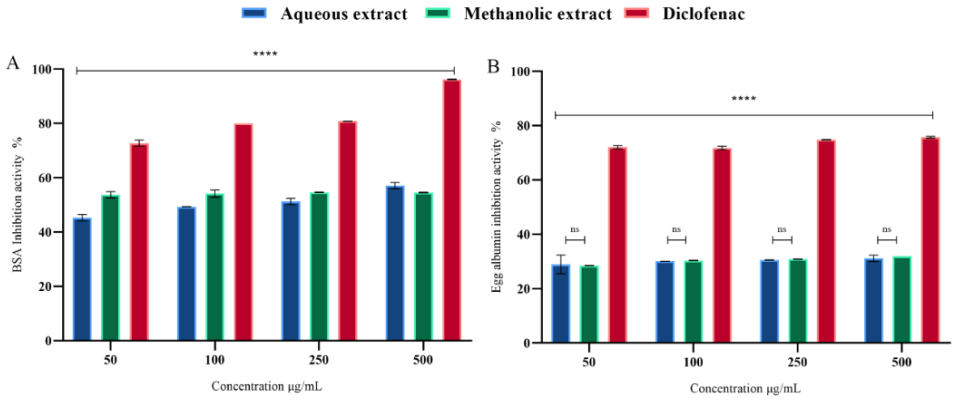

Figure 4. Inhibition of protein denaturation: BSA (**A**) and egg albumin (**B**) of *E. alata* extracts compared to s-DCF [**** (ρ<0.0001)]

Figure 5 shows the inhibitory effect of *E. alata* extracts against proteinase activity. With different concentrations, both extracts showed weak inhibition toward protease. Indeed, at the highest concentration of 500µg/mL aqueous and methanolic caused an inhibition percentage of 4.92 ± 0.07 and 3.53 ± 0.26%, respectively (p≥0.05), compared to 51 ± 0.10% inhibition by diclofenac (p≤0.05). No earlier research nor our previous study have reported the proteinase inhibition activity of *E. alata*. These low levels of protease inhibition suggest that the latter is either not involved or only minimally involved in the anti-inflammatory effects of *E. alata* extracts. Nonetheless, it has been reported that the flavonoids, abundant in our extracts, can suppress both enzymes and mediators involved in the inflammatory process by inhibition of proteinase action (Menon and Sudheer, 2007; Ribeiro et al., 2015). Notably, rutin, abundantly found in our extracts, was identified as a potential inhibitor of the SARS-CoV-2 main protease (M^{pro}) (Agrawal et al., 2021).

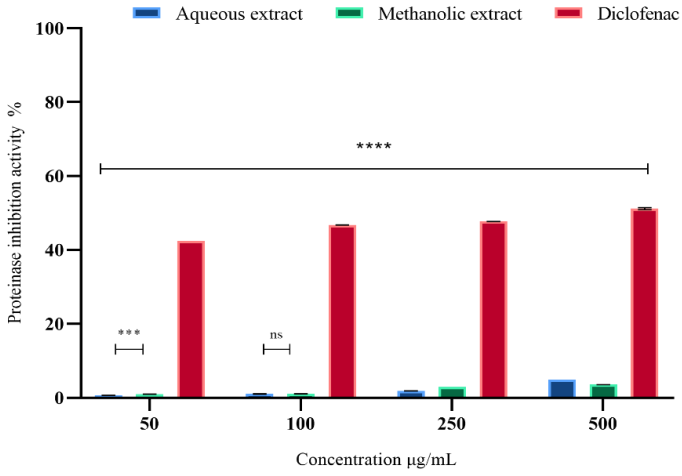

Figure 5. Proteinase inhibition activity of *E. alata* extracts compared to s-DCF

3.6. Anti-cancer Effect

The cytotoxic potential of *E. alata* was assessed on three cancer cell lines: HT-29, PC-3, and A549, which treated with increasing concentrations (25 to 200 µg/mL) of methanolic and aqueous *E. alata* extracts. As shown in Figure 6, both the methanolic and aqueous extracts exhibited weak effects, with IC_{50} values exceeding 200 µg/mL for all tested cancer and normal cell lines ($p \geq 0.05$). At the highest concentration of 200 µg/mL, a modest dose-dependent decrease in cell viability was observed in the two cancerous cell lines: HT-29, and PC-3 treated with methanolic and aqueous extracts (200 µg/mL) to 55.55, 62.61, 61.87 and 93.04%, respectively. Moreover, no significant cytotoxic effect was observed against A549 and CCD18-Co cell lines. The IC_{50} values exceeding 200 µg/mL reveal that *E. alata* extracts in the present study lack cytotoxic activity against the tested cell lines, especially when compared to typical cytotoxicity thresholds of IC_{50} below 100 µg/mL.

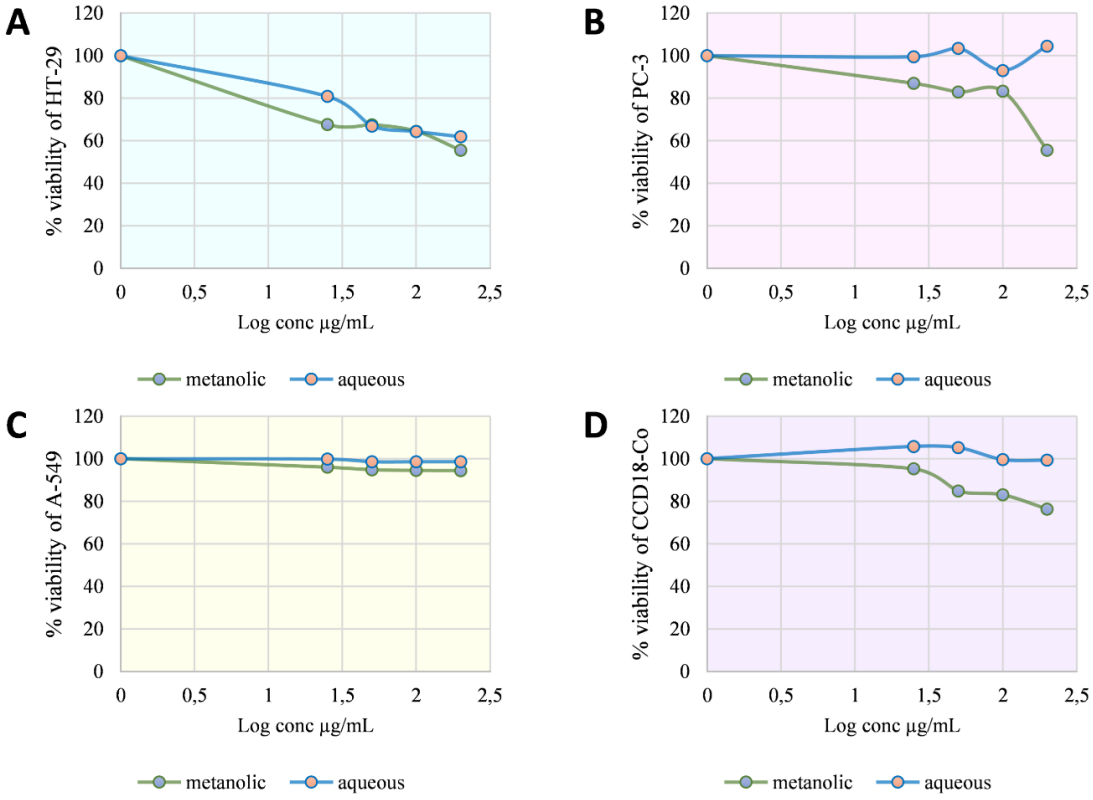

Figure 6. Cytotoxic activity of *E. alata* extracts (0 to 200 µg/mL) incubated for 72 h of treatment

In contrast, previous studies using ethanolic or ethyl acetate extracts of *E. alata* reported significantly higher cytotoxic effects. Indeed, Bensam et al. (2023) found that Algerian *E. alata* ethanolic extract inhibited the growth of the liver (HepG2), breast (MCF-7), and colon cancer cells (Caco-2). The strongest cytotoxic effect was exerted against MCF-7 cells (IC_{50} value 153 µg/mL). Additionally, the same extract induced cell death in all human cancer cells mainly through apoptosis induction (Bensam et al., 2023). Moreover, Mohammed and Mohammed (2023) screened this effect on human breast cancer cell lines (MDA-MB-231) and normal human lymphocytes (HLs) showing 50.11% cell death at a concentration of 75mg/mL on MDA-MB-231 cell lines while there was no risk in using *Ephedra* species due to its low cytotoxicity on HLs normal cell line (Mohammed and Mohammed 2023). In the same context, Danciu et al. (2018) reported that hydroalcoholic extract of *E. alata* exhibited strong anticancer activity

against MCF-7 human breast cancer cell line reaching 56.45 ± 3.9 growth inhibition at the highest concentration of 30 µg/mL (Danciu et al., 2018).

Other research investigated the antiproliferative activity of two species from *Ephedra* extracts also against MCF-7 cells via MTT and resazurin assays. The ethyl acetate extract of *E. alata* showed the highest cytotoxic activity with IC_{50} (MTT) value 26 and IC_{50} (resazurin) value 16 µg/mL while the second species *E. fragilis* had a lower effect (Soumaya et al., 2020). Nonetheless, similar results were reported for other species such as *Cassia sieberiana* and *C. singueana* (Jibril et al., 2025). These recorded discrepancies could be explained by differences in the polarity of the extracts, solvents, and extraction methods, concentrations of active compounds, and sensitivity of the cell lines tested. The relative resistance of treated cell lines to crude plant extracts could also explain this observation, which justifies the tendency of our research group to test mixtures for a more pronounced synergistic effect (Belhouala et al., 2024). This is consistent with the results of Mohammed and Mohammed (2023), who obtained significant cytotoxicity against breast cancer cells only at very high concentrations reaching 75 mg/mL, while no cytotoxicity was observed in normal human lymphocytes.

4. CONCLUSION

In the present study, we highlight notable antioxidant and anti-inflammatory properties of *E. alata* aqueous and methanolic extracts of the aerial parts, with better performance shown by the methanolic extract in several assays. We found that the methanolic extract exhibited the highest reducing power of 2.15 mg AAE/g, the strongest H_2O_2 scavenging ability of 58% that, slightly exceeded that of ascorbic acid. Interestingly, both the methanolic and aqueous extracts exhibited important HRBC membrane stabilization of 42.80 ± 0.12 and 29.34 ± 0.09%, which were 117% and 49% higher than that of the anti-inflammatory standard drug Diclofenac, respectively. These activities could be attributed to the presence of phenolic compounds such as rutin, anillin, p-hydroxy benzoic acid, pyrocatechol, luteolin, and quercetin as revealed by HPLC-DAD analysis. Nonetheless, both extracts exerted limited cytotoxic effects with IC_{50} values higher than 200 µg/mL, suggesting a lack of important anticancer activity under the tested conditions. This fact may be attributed to the polarity of the extracts, solvents and extraction methods, concentrations of active compounds, and sensitivity of the cell lines tested. However, the extracts were not cytotoxic towards normal cells, which may support their safety profile. Despite the absence of strong anticancer activity, our findings corroborate the need for further research to investigate the use of *E. alata* as a source of potent bioactive antioxidant and anti-inflammatory compounds. Furthermore, additional studies should be undertaken to demonstrate the antioxidant and anti-inflammatory potential of *E. alata in vivo,* identify molecules that could be responsible for these activities, clarify the molecular pathways involved, and investigate the possible synergistic interactions either among plant phytochemicals or between the plant-based products and conventional drugs to better manage oxidative stress, inflammatory conditions or even to serve as adjuvants in cancer therapy.

Conflict of Interest
The authors declare no conflicts of interest.

Author Contribution Statement
Conceptualization, Bachir Benarba.; methodology, Bachir Benarba.; software, Khadidja Belhouala.; validation, Bachir Benarba., Khadidja Belhouala. and Mehmet Emin Duru.; formal analysis, Khadidja Belhouala., Cansu Korkmaz.; investigation, Khadidja Belhouala., Cansu Korkmaz., Meltem Taş Küçükaydın, Selçuk Küçükaydın; resources, Bachir Benarba., Khadidja Belhouala., Mehmet Emin Duru.; data curation, Khadidja Belhouala.; writing original draft preparation, Khadidja Belhouala.; writing, review and editing, Bachir Benarba., Khadidja Belhouala.; visualization, Bachir Benarba.,

Khadidja Belhouala.; supervision, Bachir Benarba.; project administration, Bachir Benarba.; funding acquisition, Bachir Benarba, Mehmet Emin Duru. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement
The authors confirm that the data supporting the findings of this study are available within the article.

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REFERENCES

Abourashed EA, El-Alfy AT, Khan IA, Walker L. (2003). *Ephedra* in perspective - a current review. *Phytotherapy Research*, 17(7), 703-712. doi:10.1002/ptr.1337

Agrawal PK, Agrawal C, Blunden G. (2021). Rutin: a potential antiviral for repurposing as a SARS-CoV-2 main protease (Mpro) inhibitor. *Natural Product Communications*, 16(4), 1934578X2199172. doi:10.1177/1934578x21991723

Ahmed D, Fatima K, Saeed R. (2014). Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissa opaca* roots and its fractions in different solvents. *Antioxidants*, 3(4), 671-683. doi:10.3390/antiox3040671

Alqethami A, Aldhebiani AY. (2021). Medicinal plants used in Jeddah, Saudi Arabia: Phytochemical screening. *Saudi Journal of Biological Sciences*, 28(1), 805-812. doi:10.1016/j.sjbs.2020.11.013

Al-Rimawi F, Abu-Lafi S, Abbadi jehad, Alamarneh AAA, Sawahreh RA, Odeh I. (2017). Analysis of phenolic and flavonoids of wild ephedra alata plant extracts by LC/PDA and LC/MS and their antioxidant activity. *African Journal of Traditional Complementary and Alternative Medicines*, 14(2), 130-141. doi:10.21010/ajtcam.v14i2.14

Azeem AK, C. Dilip, SS Prasanth, Hanan J, Kumsr Sajeev, C. Naseera. (2010). Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*. *Asian Pacific Journal of Tropical Medicine*, 3(10), 794-796. doi:10.1016/s1995-7645(10)60190-3

Belhouala K, Benarba B. (2021). Medicinal plants used by traditional healers in Algeria: a multiregional ethnobotanical study. *Frontiers in Pharmacology*, 12, 760492. doi:10.3389/fphar.2021.760492

Benarba B, Douad O, Gadoum C, Belhouala K, Mahdjour S. (2021). Phytochemical profile, antioxidant and anti-inflammatory activities of *Ephedra alata* Decne growing in South Algeria. *Preprints*, doi:10.20944/preprints202108.0296.v1

Benarba B. (2016). Medicinal plants used by traditional healers from South-west Algeria: an ethnobotanical study. *Journal of Intercultural Ethnopharmacology*, 5(4), 320. doi:10.5455/jice.20160814115725

Bensam M, Rechreche H, Abdelwahab AE, Abu-Serie MM, Ali SM. (2023). The role of Algerian *Ephedra alata* ethanolic extract in inhibiting the growth of breast cancer cells by inducing apoptosis in a p53- dependent pathway. *Saudi Journal of Biological Sciences*, 30(6), 103650. doi:10.1016/j.sjbs.2023.103650

Bikoro Bi Athomo A, Engozogho Anris SP, Safou-Tchiama R, Santiago-Medina FJ, Cabaret T, Pizzi A, Charrier B. (2018). Chemical composition of African mahogany (*K. ivorensis* A. Chev) extractive and tannin structures of the bark by MALDI-TOF. *Industrial Crops and Products*, 113, 167-178. doi:10.1016/j.indcrop.2018.01.013

Bousenna A, Bahri F, Bouyahyaoui A, Kouidri M, Meziane M. (2022). Screening of phytochemical, evaluation of phenolic content, antibacterial and antioxidant activities of *Ephedra alata* from the Algerian Sahara. *Journal of Applied Biological Sciences*, 16(2), 220-229.

Brand-Williams W, Cuvelier ME, Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1), 25-30. doi:10.1016/s0023-6438(95)80008-5

Carocho M, Ferreira ICFR. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, 51, 15-25. https://doi.org/10.1016/j.fct.2012.09.021.

Çayan F, Deveci E, Tel-Çayan G, Duru ME. (2020). Identification and quantification of phenolic acid compounds of twenty-six mushrooms by HPLC-DAD. *Journal of Food Measurement and Characterization*, 14(3), 1690-1698. doi:10.1007/s11694-020-00417-0

Chebouat E, Hadef D, Chebouat I, Belfar ML. (2023). Antioxidant activity, phenolic and flavonoid contents of ephedra alata in southeast algeria. *Tobacco Regulatory Science*, 9(1), 251-259.

Chouikh A. (2020). Phytochemical profile, antioxidant, analgesic and hypolipidaemic effects of *Ephedra alata* decne. Female cones extract. *Farmacia*, 68(6), 1011-1020. doi:10.31925/farmacia.2020.6.7

Cowan MM. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564-582. doi:10.1128/cmr.12.4.564

Danciu C, Muntean D, Alexa E, Farcas C, Oprean C, Zupko I, Bor A, Minda D, Proks M, Buda V, Hancianu M. (2018). Phytochemical characterization and evaluation of the antimicrobial, antiproliferative and pro-apoptotic potential of *Ephedra alata* Decne. hydroalcoholic extract against the MCF-7 breast cancer cell line. *Molecules*, 24(1), 13. doi:10.3390/molecules24010013

Dbeibia A, Taheur FB, Altammar KA, Haddaji N, Mahdhi A, Amri Z, Mzoughi R, Jabeur C. (2022). Control of Staphylococcus aureus methicillin resistant isolated from auricular infections using aqueous and methanolic extracts of *Ephedra alata*. *Saudi Journal of Biological Sciences*, 29(2), 1021-1028. doi:10.1016/j.sjbs.2021.09.071

Deveci E, Tel-Çayan G, Duru ME, Öztürk M. (2019). Phytochemical contents, antioxidant effects, and inhibitory activities of key enzymes associated with Alzheimer's disease, ulcer, and skin disorders of *Sideritis albiflora* and *Sideritis leptoclada*. *Journal of Food Biochemistry*, 43(12), e13078. doi:10.1111/jfbc.13078

Hosu A, Cristea V-M, Cimpoiu C. (2014). Analysis of total phenolic, flavonoids, anthocyanins and tannins content in Romanian red wines: Prediction of antioxidant activities and classification of wines using artificial neural networks. *Food Chemistry*, 150, 113-118. doi:10.1016/j.foodchem.2013.10.153

Jaradat N, Dacca H, Hawash M, Abualhasan MN. (2021). Ephedra alata fruit extracts: phytochemical screening, anti-proliferative activity and inhibition of DPPH, α-amylase, α-glucosidase, and lipase enzymes. *BMC Chemistry*, 15(1), 41. doi:10.1186/s13065-021-00768-9

Jaradat N, Hussen F, Al Ali A. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata Decne. *Journal of Materials and Environmental Science*, 6(6), 1771-1778.

Jibril S, Sirat HM, Basar N, Hamdan S, Baba S, Muhammad A, Al-Moubarak AO. (2025). Cytotoxicity, wound healing and chemical constituents of methanol leaf extract from *Cassia sieberiana* DC and *Cassia singueana* Del. (Fabaceae). *Journal of Science and Mathematics Letters*, 13(1), 61-69. doi:10.37134/jsml.vol13.1.7.2025

Khattabi L, Boudiar T, Bouhenna MM, Chettoum A, Chebrouk F, Chader H, Lozano-Sánchez J, Segura-Carretero A, Nieto G, Akkal S. (2022). RP-HPLC-ESI-QTOF-MS qualitative profiling, antioxidant, anti-enzymatic, anti-inflammatory, and non-cytotoxic properties of *Ephedra alata* Monjauzeana. *Foods*, 11(2), 145-145. doi:10.3390/foods11020145

Kose K, Dogan P. (1995). Lipoperoxidation induced by hydrogen peroxide in human erythrocyte membranes. 1. protective effect of *Ginkgo biloba* extract (EGb 761). *Journal of International Medical Research*, 23(1), 1-8. doi:10.1177/030006059502300101

Lim EJ, Kang HJ, Jung HJ, Song YS, Lim CJ, Park EH. (2008). Anti-angiogenic, anti-inflammatory and anti-nociceptive activities of vanillin in ICR mice. *Biomolecules and Therapeutics*, 16(2), 132-136. doi:10.4062/biomolther.2008.16.2.132

Mahmoudi M, Boughalleb F, Maaloul S, Mabrouk M, Abdellaoui R. (2023). Phytochemical screening, antioxidant potential, and LC–ESI–MS profiling of *Ephedra alata* and *Ephedra altissima* seeds naturally growing in Tunisia. *Applied Biochemistry and Biotechnology*, 195(10), 5903-5915. doi:10.1007/s12010-023-04370-8

Makni M, Chtourou Y, Fetoui H, Garoui EM, Boudawara T, Zeghal N. (2011). Evaluation of the antioxidant, anti-inflammatory and hepatoprotective properties of vanillin in carbon tetrachloride-treated rats. *European Journal of Pharmacology*, 668(1-2), 133-139. doi:10.1016/j.ejphar.2011.07.001

Marsoul A, Ijjaali M, El-Hajjaji F, Taleb M, Salim R, Boukir A. (2020). Phytochemical screening, total phenolic and flavonoid methanolic extract of pomegranate bark (*Punica granatum* L): Evaluation of the inhibitory effect in acidic medium 1 M HCl. *Materials Today: Proceedings*, 27, 3193-3198. doi:10.1016/j.matpr.2020.04.202

Menon VP, Sudheer AR. (2007). Antioxidant and anti-inflammatory properties of curcumin. The molecular targets and therapeutic uses of curcumin in health and disease, *Advances in Experimental Medicine and Biology*, 595, 105-125. doi:10.1007/978-0-387-46401-5_3

Mohammed LZ, Mohammed RK. (2023). Cytotoxic activity of *Ephedra alata* extracts on human lymphocytes and breast cancer cell line in vitro. *Iraqi Journal of Science*, 64(7), 4210-4222. doi:10.24996/ijs.2023.64.7.12

Mohd WZ, Zulpahmi ND, Zukaimi NF, Musa SA, Hamid NA, Ramli NW. (2023). Antioxidant potential of different parts of three pineapple varieties N36, Madu and MD2. *Journal of Science and Mathematics Letters*, 11(2), 74-82. doi:10.37134/jsml.vol11.2.9.2023

Ngamwonglumlert L, Devahastin S, Chiewchan N. (2017). Natural colorants: Pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. *Critical Reviews in Food Science and Nutrition*, 57(15), 3243-3259. doi:10.1080/10408398.2015.1109498

Oktay M, Gülçin İ, Küfrevioğlu Ö. (2003). Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT-Food Science and Technology*, 36(2), 263-271. doi:10.1016/s0023-6438(02)00226-8

Olalekan MA. (2023). Comparative phytochemical and antioxidant analysis of the leaf extracts of two Nigerian medicinal plants. *Journal of Science and Mathematics Letters*, 11(1), 30-8. doi:10.37134/jsml.vol11.1.4.2023

Oyaizu M. (1986). Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, 44(6), 307-315. doi:10.5264/eiyogakuzashi.44.307

Oyedapo OO, Famurewa AJ. (1995). Antiprotease and membrane stabilizing activities of extracts of *Fagara zanthoxyloides*, *Olax Subscorpioides* and *Tetrapleura tetraptera*. *International Journal of Pharmacognosy*, 33(1), 65-69. doi:10.3109/13880209509088150

Ozcan T, Akpinar-Bayizit A, Yilmaz-Ersan L, Delikanli B. (2014). Phenolics in Human Health. *International Journal of Chemical Engineering and Applications*, 5(5), 393-396. doi:10.7763/ijcea.2014.v5.416

Plumb JA. (1999). Cell sensitivity assays: the MTT assay. *Cytotoxic Drug Resistance Mechanisms*, 1999, 25-30. doi:10.1385/1-59259-687-8:25

Ramirez R, Pérez-Colmenares A, Obregón-Díaz Y, Rojas-Fermín L, Cordero Y, De Lima W. (2024). Antibacterial and antioxidant activities of fruits of *Solanum hirtum* Vahl. from Venezuela. *Journal of Science and Mathematics Letters*, 12(1),102-10. doi:10.37134/jsml.vol12.1.12.2024

Ribeiro D, Freitas M, Tomé SM, Silva, Laufer S, José LFCL, Fernandes E. (2015). Flavonoids inhibit COX-1 and COX-2 enzymes and cytokine/chemokine production in human whole blood. *Inflammation*, 38(2), 858-870. doi:10.1007/s10753-014-9995-x

Ruch RJ, Cheng S, Klaunig JE. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10(6), 1003-1008. doi:10.1093/carcin/10.6.1003

Sakat S, Juvekar AR, Gambhire, MN. (2010). In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(1), 146-155.

Salleh WMNHW, Ahmad F. (2016). Antioxidant and anticholinesterase activities of essential oil of *Alseodaphne peduncularis* Meisn. *Turkish Journal of Pharmaceutical Sciences*, 13(3), 347-350. doi:10.4274/tjps.2016.11

Salleh WMNHW, Khamis S. (2020). Chemical composition and anticholinesterase inhibitory activity of *Pavetta graciliflora* Wall. ex Ridl. essential oil. *Zeitschrift fur Naturforschung C Journal of Biosciences*, 75(11-12), 467-471. doi:10.1515/znc-2020-0075

Shahidi F, Ambigaipalan P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects: a review. *Journal of Functional Foods*, 18, 820-897. doi:10.1016/j.jff.2015.06.018

Shinde UA, Kulkarni K, Phadke AS, Nair A, Aa M, Dikshit VJ, Saraf MN. (1999). Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. wood oil. *Indian Journal of Experimental Biology*, 37(3), 258-261.

Shukla S, Mehta A. (2015). Anticancer potential of medicinal plants and their phytochemicals: a review. *Brazilian Journal of Botany*, 38(2), 199-210. doi:10.1007/s40415-015-0135-0

Sioud F, Ben Toumia Imene, Lahmer A, Rihab Khlifi, Zaineb Dhaouefi, Mouna Maatouk, Kamel Ghedira, Chekir-Ghedira L. (2020). Methanolic extract of Ephedra alata ameliorates cisplatin-induced nephrotoxicity and hepatotoxicity through reducing oxidative stress and genotoxicity. *Environmental Science and Pollution Research*, 27(11), 12792-12801. doi:10.1007/s11356-020-07904-3

Sofowora A. (1993). Medicinal plants and traditional medicinal in Africa. 2nd Ed.Sunshine House. Ibadan, Nigeria.

Soumaya B, Yosra E, Rim BM, Sarra D, Sawsen S, Sarra B, Kamel M, Wissem A-W, Isoda H, Wided M-K. (2020). Preliminary phytochemical analysis, antioxidant, anti-inflammatory and anticancer activities of two Tunisian *Ephedra* species: *Ephedra alata* and *Ephedra fragilis*. *South African Journal of Botany*, 135, 421-428. doi:10.1016/j.sajb.2020.09.033

Sunmathi D, Sivakumar R, Ravikumar, K. (2016). In vitro anti-inflammatory and antiarthritic activity of ethanolic leaf extract of *Alternanthera sessilis* (L.) R.Br. ex DC and *Alternanthera philoxeroides* (Mart.) Griseb. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 5, 109-115.

Tel-Çayan G, Deveci E, Çayan F, Molo Z, Duru ME, Yeşil Y. (2021). Chemometrics evaluation of phytochemicals and antioxidant activities of the extracts of *Chaerophyllum bulbosum* roots and aerial parts. *Analytical Letters*, 55(2), 327-342. doi:10.1080/00032719.2021.1930026

Trease GE, Evans WC. (2002). Pharmacognosy. 15th Ed; London: Saunders Publishers.

Yadav RNS, Agarwala M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3(12), 10-14.

Ziani BE, Barros L, Boumehira AZ, Bachari K, Heleno SA, Alves MJ, Ferreira IC. (2018). Profiling polyphenol composition by HPLC-DAD-ESI/MSn and the antibacterial activity of infusion preparations obtained from four medicinal plants. *Food & Function*, 9(1), 149-159. doi:10.1039/c7fo01315a

Ziani BE, Heleno SA, Bachari K, Dias MI, Dalva M, Isabel CFR, Ferreira. (2019). Phenolic compounds characterization by LC-DAD- ESI/MSn and bioactive properties of *Thymus algeriensis* Boiss. & Reut. and *Ephedra alata* Decne. *Food Research International*, 116, 312-319. doi:10.1016/j.foodres.2018.08.041

Zohdi RM, Adli MA, Miskan AM, Eshak Z, James RJ, Bakar SI, Payaban M. (2025). Chemical profile, physicochemical properties, and antioxidant activity of Malaysian propolis: insights from honeybee and stingless bee. *Journal of Science and Mathematics Letters*, 13(1),1-9. doi:10.37134/jsml.vol13.1.1.2025