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Phytochemical Profile, Antioxidant and Anti-inflammatory Activities of Algerian *Ephedra alata*

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Abstract
The aim of this study was to investigate phytochemicals, the antioxidant, anti-inflammatory, hemolysis assay of Ephedra alata Qualitative analysis of phytochemicals and quantitative analysis of total phenolics and flavonoids were prepared by using standard protocols. Qualitative phytochemical analysis revealed that the aqueous extract show richness in flavonoids, terpenoids, saponins, steroids, reducing compound, tannins, phenols and alkaloids. Total phenol and flavonoid content show highest concentration in aqueous extract of E. alata (68.009mg GA Eq/gm, 4mg QEq/gm). In vitro, the DPPH and FRAP antioxidant activity, anti-inflammatory, Hemolysis assay studies show that aqueous extract of Ephedra alata showed higher antioxidant activity property and important anti-inflammatory. The results conclude that Ephedra alata contains anti-inflammatory and antioxidant compounds which protects cells against degenerative effects of Reactive Oxygen Species (ROS). This study showed that the Ephedra alata is valuable source of natural agents beneficial for human health.

Keywords: Ephedra alata, phytochemical, Antioxidant, anti-inflammatory, TPC, TFC

Introduction

Medicinal plants are receiving much attention nowadays to meet the public concern of replacing synthetic medicine with those from natural origins. The growing problem of the appearance of new diseases rises up the necessity for finding replacements from natural origins (Ghanem et al., 2008). Medicinal plants have always been important in many fields worldwide and contain certain contain various types of bioactive compounds with multiple therapeutics effects (Saidi et al., 2022). Herbs as medicines are well known and are used since long for treating various ailments. The first ever such use for any herb has been recorded by Egyptians and Ancient Chinese in medical prescription as early as 2500 BC (Dhamen et al., 2019). In addition to the therapeutic use of medicinal plants is very present in some countries of the world and especially developing countries. The inventory carried out by the WHO in the late 1970s estimated that the number of species with medicinal properties was around 21,000 worldwide. One such herb is Ephedra is a genus of non-flowering seed plants belonging to the Ephedraceae family (Hadjadj et al., 2020), which includes approximately 69 species, mainly in the desert areas of Asia, America, Europe and North Africa. Among these species, 15 ones and four varieties can be found in China (González-Juárez et al., 2020). *Ephedra alata* is a member of the genus Ephedra and is a gymnosperm, *E. alata* is a perennial shrub, stiff, yellow- green, densely branched, 40–100 cm tall and often wider than high. Twigs striate. Cones sessile and clustered in the axils (Wafa'a et al., 2010). *E. alata* is used in traditional medicine as a stimulant, antifungal and to treat allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, and headaches (Soumaya et al., 2020), to treat kidney, as well as used for treatment of cancer also the plant stems are chewed for treatment of bacterial and fungal infections (Boulaares et al., 2024). Moreover, Ephedra can also be used to treat COVID-19 infections to improve the symptoms (Tang et al., 2023). The medicinal value of plants lies in some chemical active substances that produce define physiological action on the human body

(Yadav et al., 2017). In this study, we investigate the phytochemicals, antioxidant, anti-inflammatory activities of Algerian *Ephedra alata*.

Materials and methods

Chemicals and reagents

All chemicals used were of analytical grade and purchased from Sigma-Aldrich, Mo, USA**.**

Collection, identification and extraction of plant material

The aerial part of Ephedra alata was collected in 14 November 2022 from khouinza District, El-Oued Province, Algeria. The plant material was washed using water, dried at room temperature, grounded into powder, and then stored at room temperature until use. to extract it About 50 g of the aerial parts powder of (Ephedra alata) was soaked in 500 ml of distilled water and kept at room temperature in the dark for 24 h. Then, it was filtered through filter paper. After extraction, the water was removed first using a rotary evaporator and then incubated at 60°C to dry completely. The extract was weighed and stored in a refrigerator at 4°C (Murugan et al., 2014).

Figure 1. *Ephedra alata* L.

Phytochemical Screening

The methods of qualitative phytochemical analysis as described in study of Derouiche et al (2017) (Derouiche et al., 2017) were used to identify the phytochemicals provides in the extracts: phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compond and alkaloids.

Estimation of Total Phenol

The polyphenols are determined by the Folin-Ciocalteu method. This method, initially described by Slinkard and Singleton (Slinkard et al., 1977), makes it possible to know the total polyphenolic content of a given sample**.** The sample of the aqueous extract of the E. alata (0.5 ml) and 2 ml of sodium carbonate (75 g / l) were added to 2.5 ml of 10% (v / v) Folin- Ciocalteau with gallic acid as standard. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm. The tests were carried out three times in order to ensure the reproducibility of the results. The total phenolic content was expressed in mg Equivalent of Gallic Acid per gram of sample**.**

Estimation of Total Flavonoids

Determination of the total flavonoid content of the aqueous extract of the E. alata is carried out by the method described by Derouiche et al (2022) (Derouiche et al., 2022). 0.5 ml of a 2% $AlCl_3$-ethanol solution was added to 0.5 ml of sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as a standard for plotting the calibration curve. The tests were carried out three times in order to ensure the reproducibility of the results. The results were expressed in milligram equivalent Quercetin per gram of sample**.**

Antioxidant activity (DPPH)

The in vitro antioxidant activity to *E. alata* was evaluated by measuring the scavenging power of the DPPH (1,1-diphenyl-2-picryhydrazyl) radical according to the method described by Burits and Bucar (Burits et al., 2000), where 3ml of various concentrations (25,50,100,200,400, 800,1000,1200,1400,1600,1800 et 2000µg/ml) of *E. alata* samples were added to 75µL of methanolic solution of DPPH (1.3mg/ml) . Absorbance measurements were read at 517 nm after 30 min of incubation time at room temperature (A1). Absorbance of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control (A0). The percentage inhibition [(A0-A1/A0) × 100] was plotted against the phenol content and IC50 was determined.

Antioxidant activity (FRAP assay)

The Antioxidants are determined to *E. alata* by colorimetry. The ferric-tripyridyltriazine complex is reduced to the ferrous-tripyridyltriazine in presence of the antioxidants; the complex loses its

yellow color to a dark blue. This coloration measured at 595 nm is proportional to the concentration of antioxidants present in the samples. The method is standardized by Trolox (Oyaizu, 1986; Lim et al., 2007). were Taken 500µl of sample, then we Add to it 1.25ml of the buffer solution (0.2 M, PH = 6.6), then The mixture were incubated 20 min in a water bath at 50 ° C, after that we Add 1.25ml of the aqueous TCA solution (10%) to stop the reaction and put it in Centrifugation at 3000 rpm for 5 minutes. 1.25 ml of supernatant are then mixed with 1.25 ml distilled water and 250 µl $FeCl_3$ (0.1%).Finally was measured at 700 nm against a blank. The results expired by IC50, after calculating of the ferric reducing antioxidant power values according to (Yazdani, et al., 2019) as follows:

FRAP (%) = 100 – (OD control /OD sample) × 100

Anti-inflammatory activity

The anti-inflammatory activity is measured of protein denaturation inhibition in presence of the anti-inflammatory compound, which is studied through in vitro assay. The measured turbidity at 660 nm is proportional to the concentration of anti-inflammatory compound present in the sample (Chetehouna et al., 2020). has been added different concentrations (10–100 µg ml^{-1}) of the sample to bovine serum albumin (BSA) solution (1%), then The mixture were incubated 30 min at room temperature. The pH of the solution was adjusted to 2 using dropwise addition of concentrated HCl. After incubation, the mixture is heated at 72 °C for 30 min, Finally all tubes were cooled for 10min and the turbidity was measured at a wavelength of 660nm. Diclofenac was used as standard. The results expired by IC50, after calculating of inhibition percentage (IP) as follows:

IP (%) = [(Acontrol –Asample)**/** Acontrol] × 100

where A control is the absorbance of the control, and A sample is the absorbance of the sample extract/standard (Vamanu and Nita, 2014).

Hemolysis assay

The Hemolysis assay to *E. alata* is done as described by (Vinjamuri et al., 2015) that determined the protective effect of the antioxidant compound presented in the sample against the membrane erythrocyte lysis which induced by 1X PBS. The detection of membrane RBCs lysis by measuring the concentration of hemoglobin in blood plasma at 540 nm by spectrophotometer. 5mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation, then done The blood centrifuged at 1000 rpm for 10 min at 40C, Plasma is removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min, after that The Washed erythrocytes were stored at 4 o C and used within 6 h for the hemolysis assay, and we Add 50 µL of 10 dilutions (100 µL Erythrocytes suspension and 900 µL 1XPBS) of erythrocytes suspension was mixed with 100 µL of test samples (20-80ng/mL), 100 µL of 1XPBS was used as a control. Finally, Reaction mixture is incubated at 370 C water bath for 60 min. where The volume of reaction mixture is made up to 1 mL by adding 850 µL of 1XPB, The reaction mixture is centrifuged at 300rpm for 3min, then The resulting hemoglobin in supernatant is measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. The percentage hemolysis is calculated as follows:

Inhibition percentage (%) = 100 – (OD sample /OD control) × 100

Statistical Analysis

The results were expressed as mean ± standard deviation (SD), calculated from duplicate determinations and the linear relationship was visually determined.

Results and Discussion

Phytochemical Screening

Preliminary phytochemical results showed (shown in Table 1) revealed the presence of a wide range of bioactive secondary metabolites including, phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compounds and alkaloids. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anti-apoptosis. Anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Yadav et al., 2011). The high

scavenging property of *E. alata* may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases (Pourmorad et al., 2006) An increase in phenols in plant tissue may enhance plant resistance to stress conditions. Furthermore, they can be a source of important antioxidants for human health: for example, caffeic and gallic acids inhibit carcinogenesis (Ertani et al., 2016). In terms of exploiting the diverse saponin structures that display anti-cancer properties, presumably by targeting a range of different metabolic pathways (Osbourn et al., 2011). They were investigated because they contain maesasaponins of which some were reported to have anti-cancer and lack haemolytic activity. which is commonly activity for most saponins (Faizal et al., 2013) Saponins has the property of precipitating and coagulating red blood cells (Okwu et Josiah, 2006). Flavonoids, the main group of polyphenol compounds, are the most effective antioxidants and were associated with a wide gamut of pharmacological effects including antimicrobial, anti-inflammatory, and inhibition of platelet aggregation (Boulaares et al., 2021). The six crude flavonoids extracts averred effective against several bacteria strains because the sites and the number of hydroxyl groups are thought to be related to the toxicity against bacteria. It is thought that the increased hydroxylation results in increased toxicity (Chebouat et al., 2014). aromatic steroids exhibit a wide range of biological activities, but mostly they likely possess anticancer, anti-inflammatory action of steroids might be effective to realive symptoms caused by inflammation. neuroprotection, and other activities (Dembitsky et al., 2018; Mardani, 2012). Steroids have been reported to have antibacterial properties (Opoku and Akoto, 2015).Tannins play a major role in various antibiotics used in treating common pathogenic strains (Mehta et al., 2021), Tannins are defined as phenolic compounds and plants secondary metabolites which have beneficial effects on protein metabolism in ruminants , decreasing rumen degradation of dietary protein and increasing absorption of amino acids in the small intestine (Hassanpour et al., 2011),whereas the inhibition potency on condensed tannin that can be considered as preservative agent for antibacterial, anti-yeast and antioxidant activities (Chetehouna et al., 2021). Terpenoids are reported to have anti-inflammatory , anti-viral , anti-malarial , inhibition of cholesterol synthesis and anti-bacterial activity (Indumathi et al., 2014), and play diverse functional roles in plants as hormones (McGarvey et al., 1995) also A wide range of terpenoids have demonstrated pharmaceutical activity against human ailments such as cancer (Roberts, 2007). Glycosides are known to lower the blood pressure (Gilani et al., 2000), cardiac glycosides induce inhibition of cell proliferation and / or cell death in several cancer cell lines (Trenti et al., 2014; Winnicka et al., 2006). Alkaloids are one of the important classes of secondary metabolites which are found to possess important biological properties like analgesic, muscle relaxant, antioxidant (Roy, 2017). These are used to help humans and have been shown to be beneficial for some life-threatening diseases, as these compounds possess bactericidal, anti-histaminic, anticancer, central nervous system stimulant and depressant, herbicidal, insecticidal and fungicidal properties (Kaur et al., 2015), it is effective in anti-lipid peroxide production and act as antioxidant ,antiradical (Pérez et al., 2003). Thus, the results obtained in this study suggest that the identified phytochemical compounds may be bioactive components and that these plants are proving to be an increasingly valuable reservoir of bioactive compounds with considerable medicinal value.

Table 1. Phytochemical composition analysis of aqueous extract of *Ephedra alata*

Phytochemical	Bark Aqueous extract of *E. alata*
phenol	+++
saponins	++
flavonoids	+++
steroids	+
tannins	++
terpenoids	+++
reducing compond	++
alkaloids	+

Phenolic Compounds

Phenolic compounds contain hydroxyl groups (-OH) that facilitate their free radical scavenging activity and act as antioxidants, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity (Khalil et al., 2013). The Total Phenolic Compounds was expressed in terms of gallic acid equivalents (mg of GAEq/gm sample) using the following

equation based on the calibration curve: Y = 0.0109x +0.1704 R2 = 0.9842 where x was the absorbance and Y was the mg GAEq/gm sample. Total phenolic contents of *E. alata* obtained from water solvent is 36.32 mg GAEq/gm . Phenolic compounds are well known as antioxidants and directed against free radicals associated with oxidative damage. Tannin and flavonoids act on the complications of diabetes by their antioxidant and anti-enzymatic properties, neutralizing the effect of free radicals and limiting the inflammatory reaction in different tissues (Zebidi et al., 2018), also Polyphenolic compounds prevent degenerative diseases such as cancer and cardiovascular disorders (Mahmoudi et al., 2023).

Total Flavonoid Content

Flavonoid shows antioxidant activity due to the presence of free -OH groups, especially 3-OH. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo (Derouiche et al., 2023). The Total Flavonoids Content was expressed in terms of Quercetin equivalents (mg of QE/gm sample) using the following equation based on the calibration curve: Y = 0.0194x +0.1042 R2 = 0.9903 where x was the absorbance and Y was the mg QEq/gm sample. Total flavonoid contents of E. alata obtained from water solvent is systems vary from 1.84 mg of QEq/gm . Flavonoids are a group of natural compounds with different phenolic structures, present in plants, are powerful antioxidants against free radicals, because they act as "radical-scavengers". This activity is attributed to their hydrogen-donating ability. Indeed, the phenolic groups of flavonoids serve as a source of a readily available "H" atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure, as The antioxidant activity of flavonoids depends on the arrangement of functional groups around the core structure. The configuration, substitution and total number of hydroxyl groups significantly affect several mechanisms of antioxidant activity, such as free radical scavenging and metal ion chelation ability (Tripoli et al.,2007; Boulaares et al., 2024). Flavonoids, the main group of polyphenol compounds, were associated with a wide gamut of pharmacological effects including antimicrobial, anti-inflammatory, anticancer activities, anti-diabetic, inhibition of platelet aggregation, coronary heart disease prevention and hepatoprotective, while some flavonoids exhibit potential antiviral activities (Mondal et al., 2020; Kumar et al., 2013).

Table 2. Total phenols and flavonoids concentration in *E. alata* aqueous extract

Compounds	**Polyphenols** (mg of GAEq/g extract)	**Flavonoids** (mg QEq/g) of extract
Aqueous Extract of *E. alata*	36.32 ± 0.17	1.84 ± 0.037

DPPH antioxidant activity

DPPH, a purple-colored, stable free radical is reduced to the yellow-colored diphenylpicrylhydrazine when antioxidants are added. The antioxidant capacity of the extracts were estimated and compared with ascorbic acid (positive control) using the stable DPPH radical (Kaouachi et al., 2018).The effect of antioxidants on DPPH radical scavenging was presumed to be due to their hydrogen donating ability. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. It is visually noticeable as a discolouration from purple to yellow. There is a direct correlation between antioxidant activities and the reducing power of the components of some plants. The results of the experiment for antioxidant activity are shown in Fig. 02, ascorbic acid had a very potent anti-radical activity compared to our extracts. Also , Our results indicate that the aqueous extract for Ephedra alata has a high activity against scavenging assay of free radical DPPH with an IC50=0.749 mg/ml. The extract reduces and discolours the DPPH radical due to their ability to yield hydrogen to the free radicals produced during peroxidation. Therefore, the antioxidants are considered to be reducers and activators of oxidants (Benine et al., 2022; Keser et al., 2015).The effective antioxidant activity shown by the results may be due to the abundance of bioactive components contained in this plant.

FRAP antioxidant activity

The reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Whereby the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of the sample. In this assay system, the presence of antioxidants causes the reduction of the Fe3+/ferricyanide complex to the ferrous form (Fe^{2+}), which is monitored by measuring the formation of Perl's Prussian blue at 700 nm (Qingming et al., 2010; Do et al., 2014) In Figure 3 and table 4, all extracts show some degrees of electron-donating capacity in a concentration-dependent manner. The results showed a high and close reducing power between ascorbic acid (positive control) and *E. alata* extract at their different

concentrations. The data presented here indicate that the marked reducing power of *E. alata* extract seem to be attributed to their antioxidant activity.

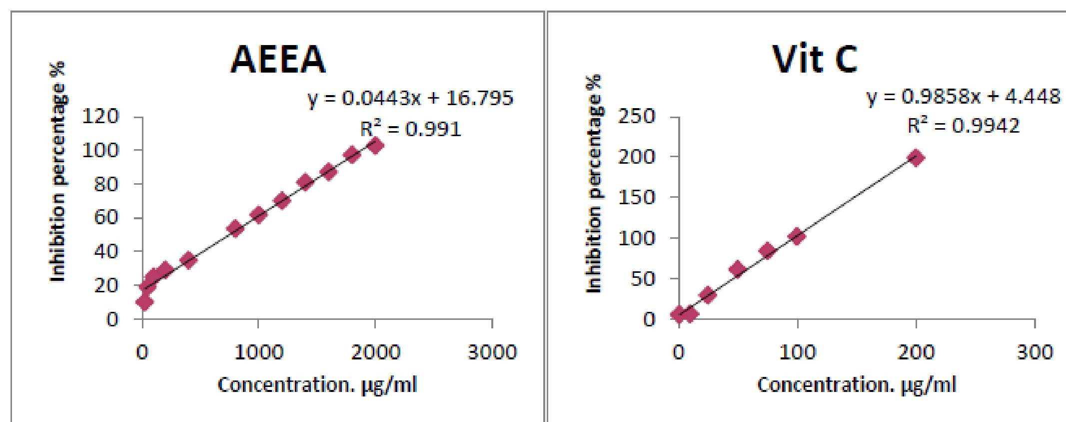

Figure 2. Inhibition percentage versus ascorbic acid and AEEA concentrations for DPPH antioxidant activity

Table 3. IC50 Value of DPPH antioxidant activity

IC50	E. alata extract	Vit C
µg/ mL	749.548	46.208

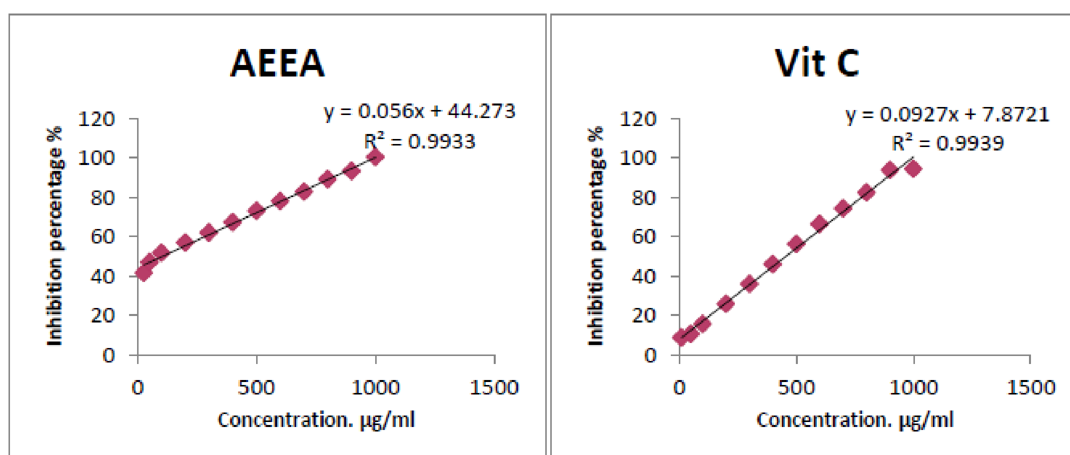

Figure 3. FRAP antioxidant activity of *Ephedra alata*

Table 4. IC50 Value of FRAP antioxidant activity

IC50	E. alata extract	Vit C
µg/ mL	102.267	454.454

Protein denaturation anti-inflammatory activity

The in vitro anti-inflammatory activity or the ability of *E. alata* to inhibit protein denaturation was studied through inhibiting heat-induced albumin denaturation (figure 4). The IC50 value against protein denaturation of *E. alata* and diclofenac was found to be 38.708 and 37.929 µg ml^{-1}, respectively. Denaturation of tissue proteins results in its loss of function and causes inflammation. Compared to the standard, diclofenac, the tested compounds have shown high anti-inflammatory activity. *E. alata* that showed efficient prevention of BSA denaturation can be further analysed for its anti-inflammatory activity in vivo (Vennila et al., 2018). The anti-inflammatory activity of *E. alata* back to presence of flavonoids in the plant (Jaradat, 2015), also this is considered the last are powerful inhibitors of the production of prostaglandins, very active pro-inflammatory molecules. Flavonoids inhibit the migration of leukocytes by blocking their adhesion to the vascular wall (Lakache et al., 2021). As This beneficial effect may be due to the presence of polyphenols in *E. alata* extracts as it has been shown, in various in vivo and in vitro studies, that polyphenols decrease markers of inflammation and acted on many molecular targets at the center of inflammation signaling pathways (Chetehounaa et al., 2024; González-Gallego et al., 2010; Santangelo et al., 2007).Many studies have shown that many flavonoids and related polyphenols contribute significantly to the anti-inflammatory activities exerted by many plants

(Marrassini et al., 2018), Terpenoids are reported to have anti–inflammatory (Indumathi et al., 2014).

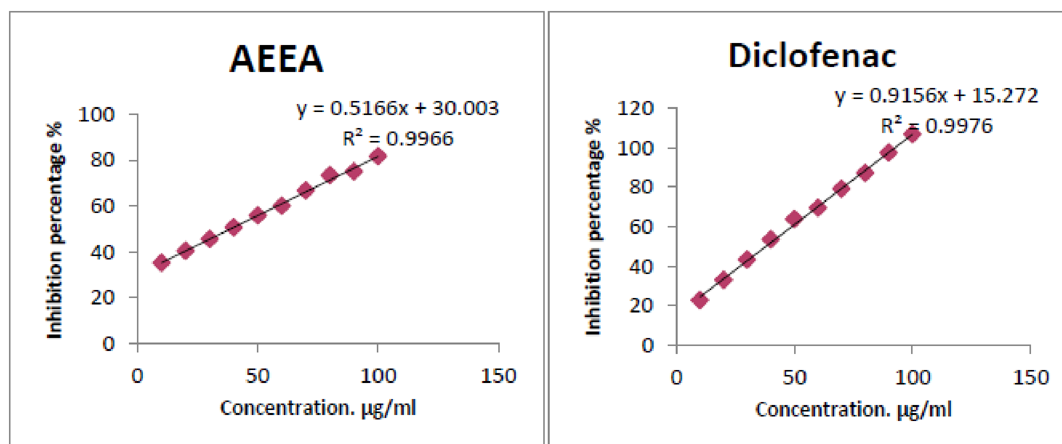

Figure 4. Inhibition percentage versus concentration of *Ephedra alata* and diclofenac for protein denaturation test

Hemolysis test

In the hemolysis test, the results are presented in figure 5. We found that extract was in High protected for the erythrocytes: Percentage of Hemolysis of extract of Ephedra alata and Ascorbic Acid. The results revealed that *Ephedra alata* contains biologically active compounds that counter the decomposition of red cells (this is mainly due to the properties of its compounds such as phenols, flavonoids, and antioxidant tannins in eliminating reactive oxygen species). That has the ability to inhibit oxidative stress. Because the rate of haemolysis is much higher when red blood cells are treated with hydrogen peroxide**.** This could be attributed to the oxidative nature of hydrogen peroxide and its ability to destroy the cell membrane and consequently the release of haemoglobin from cells **.**According to H_2O_2 cause degradation of hemoglobin in erythrocytes thus formed Fe ions generated by the reaction of the OH hydroxyl radical. The antihemolytic activity of plant extracts may be due to inhibition of the radical by the bioactive compounds in the extract which releases electrons to H_2O_2 thus neutralizing a water molecule (Chouikh, 2020; Boulaares et al., 2024).

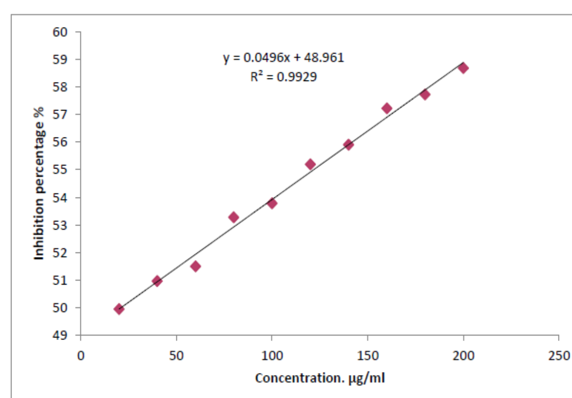

Figure 5. Hemolysis activity of *Ephedra alata*

Table 5. IC50 Value of protein denaturation and hemolyse test

	Protein denaturation test		Hemolyse test
IC50	*E. alata* extract	Diclofenac	*E. alata* extract
µg/ mL	38.70	37.92	20.94

Other studies on *Ephedra alata* Decne in both Bechar and southern Algeria showed bioactive components in the aqueous extract and compared to our study on *Ephedra alata* in El-Oued state; On the abundance of steroids, phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compond and alkaloids for *Ephedra alata* of El-Oued state, while the results showed that *Ephedra alata* Decne for the state of Bechar did not contain terpenoids and Ephedra alata Decne for southern Algeria lacked the presence of both alkaloids and glycosides and was *Ephedra alata* of El-Oued is very rich in the rest of the biologically active components compared to both types of *Ephedra alata* Decne from Bechar and Southern Algeria because it contains a lower percentage.

Therefore, *Ephedra alata* of El-Oued may have higher and more effective antioxidant and anti-inflammatory activity (Chetehouna et al., 2024; Hibi et al., 2022).

Conclusion

The phytochemical screening showed that the Ephedra alata plant extract contain a mixture of phytochemicals as phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compond and alkaloids, The quantitative total flavonoids and total phenol screening indicated that the Ephedra alata plant extract It has a high content of flavonoids and phenols and the DPPH and FRAP assay showed that the plant has potent antioxidant activity which can be an excellent choice for biological and chemical analysis , It also appeared that Ephedra alata of El Oued state is rich in secondary metabolites compared to Ephedra alata decne in both Bechar and southern Algeria, and due to its abundance of secondary metabolites, it has a high healing potential, and can be further subjected for the isolation of the therapeutically active compounds with anticancer potency. The results also showed that *E. alata* has anti-inflammatory activity and a greater protection of cells from degradation.

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