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# *Ephedra alenda* from the Algerian Sahara: Ethnobotanical Investigation, Evaluation of Total Polyphenols, Antioxidant Potential and Antibacterial Properties

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## ABSTRACT

**Background:** This study is part of the contribution to the development of the medicinal plant *Ephedra alata* from the region of Bechar, Algeria. This work aimed to measure yields, total phenols, total flavonoids, antioxidant capacity and antibacterial evaluation.

**Methods:** In this laboratory investigation during March 2023 to December 2023, the samples were macerated; total phenolic and flavonoids contents were analyzed using a colorimetric method, while antioxidant capacity was assessed through DPPH. The antibacterial essay was carried out by the disc diffusion method on a solid medium using Muller Hinton.

**Result:** Phytochemical screening confirms the presence of alkaloids, flavonoids, free quinones, reducing sugars, saponins, sterols, tannins and terpenes. Methanol extract represents the highest yield (4.41%), followed by butanol extract (3.3%), ethyl acetate extract (1.25%) and dichloromethane extract (0.69%). The dosage carried out revealed the content of plyphenols and flavonoids in the methanol extract was (175.73±0.25 µg EAG/mg DE) and (35.2±0.17 µg QE/mg DE), respectively. The evaluation of the antioxidant effect of the crude extracts by free radical scavenging DPPH gave the $IC_{50}$ values of the extracts methanol, butanol, ethyl acetate and dichloromethane (0.170, 0.244, 0.324 and 2.23 mg/mL), respectively. The investigation into antibacterial activity revealed that the methanol extract significantly inhibited all tested bacteria, while the dichloromethane extract showed the lowest inhibition of *Escherichia coli*.


**Key words:** Antibacterial, *Ephedra alata*, Flavonoids, Gallic acid equivalent, Quercetin.

## INTRODUCTION

The relationship between humans and nature has existed for centuries; it continues to evolve to meet the demands of fields such as pharmacy, medicine and beauty. Traditional medicine was prevalent among our ancestors, especially in African countries, which piqued researchers' interest in natural substances with biological efficacy, such as antioxidants and antibacterials. Medicinal plants are a significant source of highly valuable active ingredients and new molecules for industry (Chibani, 2013). Around 25% of modern medicines are currently developed from plants.

Algeria is particularly mentioned when discussing the natural wealth of medicinal plants in Africa, because it boasts a wealth of medicinal flora, with around 3,000 plant species, 15% of which are endemic. Additionally, a large proportion of the population persistently uses plants for medicinal purposes (Boudjerda, 2017).

The Sahara's flora is characterized by some of the most significant endemic species, like *Odenya africana, Zilla macroptera, Limoniastrum feei, Launaea nudicaulis*, *Bubonium graveolens, Atriplex halimus, Ephedra alata, etc* (Hammiche and Maiza, 2006; Berreghioua and Ziane, 2024).

*Ephedra* is the only genus in the *Ephedraceae* family. It comprises 50-65 species. Generally, it is abundant in dry and open habitats such as deserts, rocky slopes, grasslands and maritime areas with a Mediterranean climate (Hegazi and El-Lamey, 2011, Hegazi *et al*., 2020). In Egypt, *E. alata* is used in traditional medicine as a depurative, hypotensive, anti-inflammatory and astringent agent. In Saudi Arabia, has been used as pasture for many animals attracted by its acceptable aroma (Brullo *et al*., 2022). In Algeria, *E. alata* is used to treat flu, whooping cough and general weakness. It is consumed as an herbal tea or through inhalation and nasal drops are used to combat colds (Ghourri *et al*., 2013). In India, it is used to relieve bronchial asthma (Garla *et al*., 2011; Limberger *et al*., 2013). If consumed in high doses or over a prolonged period, the ephedrine contained in *Ephedra* can cause numerous undesirable effects, such as insomnia, nervous agitation, irritability, hypersudation, restlessness and mydriasis (El Fennouni, 2012). Most of the *in vitro* work carried out on *Ephedra* is related to alkaloids and flavonoids

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which constitute the most important category of polyphenol molecules and are very famous for their antioxidant properties (Garla *et al*., 2011).

Given these considerations and to confirm that the Saharan medicinal plants are interesting (Berreghioua and Cheriti, 2018; Bennaceur *et al.*, 2021; Berreghioua and Ziane, 2024), this study aimed to measure yields, total phenols, total flavonoids, antioxidant capacity and antibacterial evaluation of some extracts from *Ephedra alata alenda* growing in Algerian Sahara.

## MATERIALS AND METHODS

This study was conducted from March 2023 to December 2023 at the Chemistry Sciences and Environment Laboratory (CSEL), Department of Materials Sciences, Exact Sciences Faculty, University of Bechar; (Algeria). Using the global positioning system, the location of the plant from which the specimens were taken was ascertained. 31°55′24″North, 2°27′51″West, the altitude was roughly 1840 meters.

### General experimental procedure

Ultraviolet (UV) spectra had been received in methanol (MeOH) solvent with a Unicam UV300 spectrophotometer. Thin layer chromatography (TLC) was executed on silica gel 60 F254 plates (Merck, Germany).

### Plant materials and chemicals

*Ephedra alata,* known by the common name “alanda”, is widely distributed in the Algerian Sahara . It was collected in March 2023 from Boukais, (South of Bechar, south western Algeria) (Fig 1) where we found several plants and herbs. The plant was identified by several herborists. A voucher specimen is deposited under the number BA 23/1 in the herbarium of CSEL. The stems of *Ephedra alata* (L.) were dried in a dry and shady place at ambient temperature for two weeks. Finely small pieces were stored in airtight polythene bags protected from sunlight until use.

### Extractions

200 g of stems were defatted using 800 mL of hexane in a 2000 mL flask with a reflux condenser for 4 hours. The mixture was filtered and the residue was dried. 100 g of the defatted plant were exhaustively treated with MeOH (400 mL) at reflux. The rest was macerated in a mixture of ethanol and water (80/20 v/v) with stirring for 48 hours, using fresh solvent every day. After filtration, the ethanol was evaporated at 35°C using a rotary evaporator at a boiling point of 78°C to obtain an aqueous extract. After filtration, the aqueous solution was successively extracted with $CH_2Cl_2$, EtOAc and *n*-BuOH using 50 mL of each solvent three times and then the extracts were concentrated to dryness (Moussaoui *et al*., 2010).

### Estimation of total phenolics

The determination of the total polyphenols is carried out with the Folin-Ciocalteu colorimetric reagent according to the method cited by Nurcholis *et al.* (2022), with minor modifications. 50 µL of each sample (extract) was dissolved and mixed with 150 µL of the Folin-Ciocalteu reagent 10 times diluted in methanol. The mixture was stirred and incubated for 4 minutes. After, 200 µL of sodium carbonate solution $Na_2CO_3$ (7.5% w/v in distilled water) was added. The final mixture was shaken and then incubated for 2 hours in the dark at room temperature. The contents of all the extracts were measured by a UV spectrophotometer at 765 nm. The test contains all the reagents and does not contaminate the extract. The quantification of the polyphenols was made according to a linear calibration curve (y = a x + b) produced by a “gallic acid” standard with concentrations of 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL. The concentrations of the extracts were expressed in micrograms of gallic acid equivalent per milligram of dry extract (µg GAE / mg DE).

### Total flavonoids assay

With a few minor adjustments, the total flavonoid content was calculated using the Khumaida *et al*. (2019) approach. 50 µL of each extract was added to 100 µL of water, 20 µL of aluminum chloride ($AlCl_3$) (2% w/v in methanol) and 20 µL of potassium acetate ($CH_3COOK$). The mixture was stirred and left for 20 minutes in a dark room. The contents of all the extracts were measured by a UV spectrophotometer at 430 nm. The quantification of flavonoids was made according to a linear calibration curve (y= a x + b) produced by quercetin with concentrations of 0.2 mg/mL, 0.4 mg/mL and 0.6 mg/mL from 10 mg of quercetin dissolved in methanol. The concentrations of the extracts are expressed in micrograms of quercetin equivalent per milligram of dry extract (µg QE/ mg DE).

### Antioxidant activity assessment

In recent years, a great deal of attention has been focused on identifying plants with antioxidant abilities that may be used for human consumption. It was suggested to screen




**Fig 1:** The map of Algeria (position of the harvesting region) available on Algérie-monde.com.

successive extracts of *Ephedra alata* for *in vitro* antioxidant activity using standard procedures, given the various ethnobotanical uses of the plant mentioned above. The antioxidant activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, according to Nurcholis *et al.* (2022). It is based on the reduction of an alcoholic solution of the stable radical species DPPH in the presence of a hydrogen-donating antioxidant (AH). This results in the formation of a non-radical form DPPHH.

The DPPH radical was one of the first radicals used to investigate the correlation between the structure of phenolic compounds and their antioxidant activity. Modifications have been made since then and an important parameter has been introduced: The determination of $IC_{50}$. This is defined as the substrate concentration that results in a 50% reduction in absorption. At this concentration, 50% of DPPH is in its reduced form (Beddou, 2015). In order to ensure the accuracy of the results, all glassware used in this experiment was wrapped in aluminum foil, as DPPH is sensitive to light. A solution of 4 mg DPPH in 100 mL of methanol was prepared and stirred constantly for at least one hour. To create a stock solution, 2 mg of plant residue are placed in 2 mL of methanol in a series of flasks. Dilutions are then made to achieve different extract concentrations (0.5, 0.25, 0.125, 0.0625, 0.0312 and 0.0156) in micrograms per milliliter (µg/mL). Dispense 100 µL of each concentration into separate vials and add 3 mL of DPPH. Shake the vials vigorously using a vortex mixer. Incubate the vials in the dark at room temperature for 30 minutes. The reduction of DPPH- to DPPH-H causes a loss of its violet color, UV spectra have been received in MeOH solvent with a Unicam uv 300 spectrophotometer at a wavelength of 517 nm (Souhoka *et al*., 2019). Ascorbic acid' was used as a positive control at the same concentrations described above. The antioxidant activity (in%) is graphed as a function of the different extract concentrations tested to calculate the $IC_{50}$. A low $IC_{50}$ value indicates a high capacity of the extract to act as a DPPH scavenger (Bouhaddouda, 2016).

**Antibacterial investigation**

Pure cultures of the following microorganisms were used: *Escherichia coli* (ATCC 25922)*, Pseudomonas aeruginosa (*ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) which were obtained from the Pasteur institute (Algiers, Algeria). These strains were identified and purified in the microbiology laboratory at the University of Bechar in Algeria. They were maintained by frequent sub-culturing on Mueller Hinton agar plates and stored at 4°C. Mueller-Hinton agar was acquired from a reliable commercial source and the disc diffusion method was utilized to conduct the antibacterial assay. Mueller-hinton agar formulations only compliant with NCCLS document M6-P's acceptability limits (Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard) should be used (NCCLSI, 2006). Mueller-Hinton agar was made in compliance with the manufacturer's guidelines. Every Mueller-Hinton agar preparation should have its pH measured; it should be between 7.2 and 7.4. (NCCLS M31-T offers appropriate techniques to measure the pH of agar media.) Each batch of medium should be examined to ensure that it can sustain the development of the most pertinent control strains before being used (NCCLSI, 2006). After a 20-hour culture growth at 37°C, the strains were reactivated and adjusted to $10^8$ colony forming units per milliliter (CFU/mL). Ten milliliters (10 mL) of the medium Muller Hinton were put into Petri boxes (9 cm in diameter). The bacterial strains were applied in radial patches to the agar plate surface using swabs and suspensions of young bacterial cultures made in accordance with the committee for laboratory standards institute (CLSI, 2015). Due to the lack of miscibility of the majority of the extracts with water and therefore in the culture medium, a dilution was achieved by a solution of dimethyl sulfoxide (DMSO). Sterile filter paper discs with a diameter of 6 mm were used to apply the extracts to the inoculated agar surfaces. Each disc was impregnated with 10 µL of extract. A disc impregnated with DMSO was used as a negative control, while chloramphenicol (10 µg/mL) was included as a positive control. Before incubation, gently press each disc downward to ensure complete contact with the agar surface. The Petri dishes were then incubated at 37°C for 24-48 hours (Berreghioua *et al*., 2016). Results were read by measuring the inhibition diameter of a round disc using sliding calipers or a ruler. Each experiment was conducted in triplicate, with the results recorded on the back of the inverted Petri dish.

## RESULTS AND DISCUSSION

Generally, chemical components like flavonoids and phenolics were isolated from plants and natural products. The objective of extracting phenolic compounds from their plant sources is to liberate these compounds from the vacuolar structures where they are found, either through rupturing plant tissue or through a process of diffusion. The most popular techniques for removing phenolic and flavonoid components from spices include Soxhlet extraction, supercritical fluid extraction, maceration and ultrasonication (Yodi *et al*., 2023).

After analyzing the results of the phytochemical examination, it was discovered that the *Ephedra alata* species from the Bechar region is rich in secondary metabolites, such as tannins, flavonoids, alkaloids, saponins, reducing sugars and free quinones. The weight ratio of the extract to the sample weight is known as the extract yield. In our study, the yields for maceration extraction ranged from 0.69% to 4.4%. Choosing the right solvent for the maceration is crucial because it affects the phytochemicals that are recovered from the samples and makes it possible to extract thermolabile phytochemicals (Bitwell *et al*., 2023). Table 1 shows that the MeOH extract had the highest yield (4.41%), followed by the BuOH extract (3.3%), the AcOEt (1.25%) and finally the dichloromethane

extract (0.69%). The difference in chemical composition between the same plants being studied, as well as those in another region, can be attributed to various factors that affect the presence, absence and distribution of active ingredients. These factors include climate, soil type, water, altitude, *etc* (Boughrara, 2016).

The two main variables affecting the amount of extracted chemicals are the choice of extraction method and extraction time. The plant species, the organ utilized for extraction, the drying conditions, the amount of metabolites in each species and the type of the solvent all affect the extraction yield. The sample-to-solvent ratio and temperature are two other variables that affect the compound yields (Pham *et al*., 2019, Nurcholis *et al*., 2022).

One or more phenol rings, which are hydroxyl groups joined to aromatic rings and readily oxidize by giving a hydrogen atom to free radicals, define phenolic compounds, which are secondary metabolites (Shahidi *et al*., 2015); Phenolic substances are extremely powerful antioxidants because they can produce stable phenoxy radicals during oxidation processes (Ali *et al*., 2013). Phenolic chemicals are well known to be essential for scavenging free radicals, lowering oxygen concentrations and protecting and regenerating other antioxidant molecules (Aici and Benmehdi, 2020).

The Folin Ciocalteu reagent is a yellow acid consisting of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). It is reduced, during the oxidation of phenols, to a mixture of blue oxides of tungsten and molybdenum. The coloration produced, whose maximum absorption is at 760 nm, is proportional to the quantity of polyphenols present in the plant extracts (Singleton and Rossi, 1965).

The findings indicate a strong correlation between the two substances, with higher total phenolic content being correlated with higher total flavonoid content). Sometimes the true total content is not well represented by a high total flavonoids content (TFC). This is due to the possibility that chemicals detected by the colorimetric technique with $AlCl_3$ could result in higher flavonoid content. These include substances having ortho-hydroxyl carbonyl groups, like curcuminoids. These are not flavonoids, but they have similar qualities and react with the $AlCl_3$ reagent (Yodi *et al*., 2023). In recent years, the field of biological and medical sciences has introduced the concept of oxidative stress. This refers to a situation where the cell cannot control the excessive presence of toxic oxygen radicals. Oxidative stress is linked to many human diseases, including aging, cancer, atherosclerosis, cardiovascular accidents and neurodegenerative diseases (Favier, 2003; Da Silva *et al*., 2004).

In the past, new compounds with diverse qualities for humans and belonging to distinct chemical families have been inspired by plants. These molecules have intriguing biological properties that have drawn the attention of numerous researchers who hope to better understand them and progress medicine. Because of its efficacy, low toxicity, accessibility, affordability and acceptability, herbal therapy has been used extensively or exclusively by almost all cultures and civilizations from antiquity to the present (Benhammou *et al.,* 2009; Berreghioua *et al.*, 2013; Djellouli *et al.*, 2015; Vaou *et al*., 2021; Bendifallah, 2023; Berreghioua and Ziane, 2024).

The plants have developed an enzymatic and non-enzymatic defense system to prevent excessive accumulation of reactive oxygen species (ROS). This protection is based on several mechanisms of action: Direct capture of free radicals by antioxidant enzymes and/or neutralization of radicals formed by natural antioxidants; these last are molecules, either natural or synthetic, that prevent or limit the oxidation process by either reacting with free radicals to form stable species or preventing their formation altogether. Their primary function is to maintain a balance between the production of free radicals and their elimination, thereby preventing tissue and cell damage (Mboliyou, 2016).

Atoms or molecules that have an unpaired electron in one of their orbitals and are able to exist on their own are known as free radicals. Furthermore, free radicals can start a harmful chain reaction in the body since they are extremely unstable and reactive (Lobo *et al*., 2010). Because this process denatures proteins, lipids, carbohydrates and even deoxyribonucleic acid (DNA), it can result in a variety of diseases (Sharifi-Rad *et al*., 2020).

Based on the $IC_{50}$ results, the extracts of methanol, butanol and ethyl acetate showed very good activity at concentrations of 0.170, 0.244 and 0.324 mg/mL, respectively, compared to the standard ascorbic acid. The DCM extract showed moderate activity (0.850 mg/mL). As a reference antioxidant, ascorbic acid has shown a powerful capacity to scavenge DPPH-free radicals. This standard is highly effective against free radicals in biological systems. Previous studies demonstrated the antioxidant capacity of the methanol extract of *E. alata*. Therefore, this medicinal

**Table 1:** Outcomes from some *Ephedra alata* extracts.

| Extract of | Yield (%) | Total phenol (µg GAE/mgDE) | Total flavonoid (µg QE/mgDE) | $IC_{50}$ (mg/mL) |
|---|---|---|---|---|
| AcOEt | 1.25 | 157.13±0.18 | 22.21±0.20 | 0.324±0.30 |
| BuOH | 3.31 | 160.37±0.32 | 27.18±0.11 | 0.244±0.27 |
| $CH_2Cl_2$ | 0.69 | 149.31±0.25 | 19.14±0.09 | 2.23±0.34 |
| MeOH | 4.41 | 175.73±0.25 | 35.21±0.17 | 0.170±0.20 |

GAE: Gallic acid equivalent; DE: Dried extract; QE: Quercetin equivalent; IC: Inhibition concentration.

**Table 2:** Inhibition zone diameter (mm).

| | EtOAc | BuOH | $CH_2Cl_2$ | MeOH | Chloramphenicol (10 µg/mL) |
|---|---|---|---|---|---|
| *Escherichia coli* | 10 | 11 | 8 | 13 | 35 |
| *Pseudomonas aeruginosa* | 12 | 12 | 9 | 16 | 28 |
| *Staphylococus aureus* | 12 | 13 | 9 | 15 | 15 |

plant can serve as a potent source of antioxidants (Palici, 2016, Mohammedi, 2013, Achraf *et al*., 2023).

Total phenolic and flavonoid concentration has a major impact on a plant extract's antioxidant activity; higher content corresponds to a higher antioxidant capacity (Yodi *et al*., 2023). Thus, in comparison to other solvents, the high total phenolic and flavonoid content in the MeOH extract yielded the best antioxidant capacity. Conversely, the lowest DPPH reduction was produced by the lowest total phenolic and flavonoid content in the $CH_2Cl_2$ extract.

The results of the antibacterial activity tested by the disc diffusion method in agar medium on three bacterial strains are registered in Table 2.

The MeOH extract had a significant inhibitory effect on all bacteria tested, its highest activity was against *P. aeruginosa*. The presence of flavonoïds, tannins, cardinolids, saponosids and alkaloids in MeOH extract, is responsible for its high activity against the majority of bacteria.

The extracts of BuOH and MeOH were found to be highly effective against *Staphylococcus aureus*. According to Berreghioua *et al*. (2013) and Berreghioua *et al*. (2016), this bacterium is generally known to be highly resistant to various antimicrobial agents and antibiotics. It is established that inhibiting this bacterium requires considerable concentrations of antimicrobial agents. *S. aureus* is responsible for causing a number of diseases in both humans and animals. Despite advances in medical science, epidemiology and the discovery of new antibiotics, *S. aureus* infections still cause significant morbidity and mortality (Berreghioua *et al*., 2016). The dichloromethane extract did not inhibit the growth of *Pseudomonas aeruginosa*. Infections caused by *P. aeruginosa* are difficult to treat with conventional antibiotics. *Ephedra alata* has shown significant antiviral activity against the Herpes simplex virus (HSV). The aqueous extract of Egyptian *E. alata* has the potential to inhibit the growth and production of aflatoxins by *Aspergillus flavus*, both *in vitro* and *in vivo*. Ghanem and El-Magly found that the acetonitrile extract of Egyptian *E. alata* exhibited strong activity against both gram + and gram - bacteria, as well as fungi and yeast-like fungi (Ghanem and El-Magly, 2008).

## Conclusion

Most medicines today are concentrated versions of plant remedies. The aim of this study was to contribute to the valorization of *Ephedra alata alenda*, a species of the *Ephedraceae* family. The phytochemical tests revealed that *Ephedra-alata-alenda* from the Bechar region is abundant in secondary metabolites, such as tannins, flavonoids, alkaloids, saponins, reducing sugars and free quinones. The antioxidant activity of some extracts was evaluated using the DPPH method. The results indicate high activity for all extracts. These findings suggest that additional phytochemical and biological investigations are necessary for this species by isolating and purifying secondary metabolites from the extracts and analyzing them using NMR and MS techniques to identify the molecule(s) responsible for the antioxidant, antibacterial and antifungal effects, as well as any synergy between them.

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### Author contributions

Concept: Berreghioua, Ziane; Design: Berreghioua; Control: Berreghioua, Ziane; Sources: Berreghioua, Ziane; Materials: C.S.E.L.; Data Collection and Processing: Berreghioua, Ziane; Analysis and/or Interpretation: Berreghioua, Ziane; Literature Review: Berreghioua; Manuscript Writing: Berreghioua; Critical Review: Berreghioua.

### Ethics committee approval

The authors declare that ethics committee approval is not required for this study.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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