Original Research Article

Phytochemical profile, antioxidant and antibacterial activities against pathogenic bacteria of *Ephedra alata* extract

ABSTRACT

Phytochemical, antioxidant and antibacterial activities of *Ephedra alata* extract is the goal of this study, in particular medicinal plants. The phytochemical investigation, determination of polyphenols content, antioxidant and antibacterial activity of extracts from an important medicinal plant have been used in popular medicine for treating many diseases: *Ephedra alta* was carried out in this work.

According to phytochemical studies results. Numerous secondary metabolic products including such as Flavonoids, Saponins, Tannins were detected. Additionally, several secondary metabolic products such as Steroids and Cardinolids are absent. The yield for the aqueous extract was 9.89 %. However, the polyphenols extract content was equal to 0.004243 (mg GAE/ml extract.(Effectiveness of antioxidant plant using DPPH free root was estimated at IC_{50}= 0.901 mg/ml for aqueous extract. For In vitro antibacterial activity produced by the disc diffusion method, and results obtained suggest that natural substances have an important antibacterial power on multi-resistant germs such as: *Pseudomonase aeruginosa* and *Serratia fonticola* responsible of many infectious. Growth inhibition varies with bacterial species and concentration of *Ephedra alta* extract.

Keywords: Antioxidant, Antibacterial Activity, *Ephedra alata*, Penicillin, Multi-Resistant germs.

1. NTRODUCTION

Reactive oxygen species (EROs) are implicated in physiological processes at low levels. However, the primary cellular components may become poisonous if EROs synthesis is too high; lipids, proteins and nucleic acids, and cause oxidative stress which will be implicated in various pathologies such as cancers, diabetes, Inflammatory diseases... [1]. In addition, the fight against bacterial infections becomes complicated because many bacteria have developed resistance to most antibiotics, which has been a major world health challenge [2].

However, It is concerning that synthetic compounds have negative consequences when used to treat bacterial infections and oxidative stress. Also finding a replacement for the usage of chemical antioxidants and traditional antibiotics seems to be crucial. Herbal therapies are a viable alternative to conventional primary care systems, making them a promising way to the development of conventionally enhanced medications [3]. The abundance of natural substances found in medicinal plants, such as polyphenols, flavonoids, tannins, etc., that have antioxidant and antibacterial properties, has recently piqued the interest of many researchers.

Scientific research's top priorities now include finding novel bioactive compounds from natural sources that have few or no adverse effects and using them as therapeutic substitutes for manufactured molecules [4]. For this study, we investigated phytochemical compounds, antioxidant and antibacterial activity of *Ephedra alata*, an herb widely used in south Algerian traditional medicine.

2. MATERIALS AND METHODS

2.1. Materials

Plant material :

Aerial parts of *Ephedra alata* were collected from Alenda village, Wilaya of El-Oued (South-east Algeria), were properly cleaned by being washed under running water to remove dust and other foreign objects, and then shade dried for 15 days.

Bacterial strains:

The microbiological material consists of five bacterial strains. These are Gram-positive and or Gram-negative bacteria and pathogenic to humans, often multi-resistant to antibiotics and causing serious infections. They come from the El Majed laboratory (Private laboratory) located in the wilaya of El-Oued (Algeria). The bacterial strains are: *Escherichia coli, Pseudomounase aeruginosa, Staphylococcus aureus, Proteus mirabilis and Serratia fonticola*.

Antiibotic:

To compare the antibacterial effects of natural products isolated in the aqueous extract with those antibiotics, . Penicillin was used in (10 mg) [discs with a diameter of 6 mm contain 10 mg of the antibiotic].

2.2. Methods followed

Preparation of plant extract:

400 g of shade-dried powdered aerial parts was extracted by using 4 L of water. , for The resultant extract was filtered using Whatman Filter paper (N°: 1) and evaporated at low pressure, thus dried. 13% w/w was the extract yield percentage of the initial raw material and stored in refrigerator at 4 °C for further use .

Phytochemical qualitative analysis

Phytochemical analysis of the plant extract was evaluated using the following standard procedures [5,6,7,8]. Qualitative analysis were done to find the presence of the biochemical constituents such as Alkaloids, Flavonoids, Anthocyans, Saponins, Terpenoids and steroids, Leucoanthocyans, Cardinolids, Gallic tannins and Catechic tannins.

Determination of total phenolic content

Total phenolic content were determined according to method of Singleton & Rossi the literature [9]. Briefly, 200 µl of Folin-Ciocalteu reagent received added to 100 µl of the diluted sample. A saturated sodium carbonate solution (Approximately 20%) in 600 µl was added after 3 minutes. The absorbance of the sample was measured at 765 nm after 2 hours of incubation at room temperature. The standard curve was produced by applying the same process repeatedly to all standard Gallic acid solutions (0 - 100 µg/ml).

DPPH free radical scavenging assay

Of DPPH, 2.4 mg was dissolved in 100 ml of methanol to prepare make the 1,1-diphenyl-2-picrylhydrazyl solution. Of extract, 50 µl (or ascorbic acid as a control) is was added to 1.950 ml of the DPPH - solution previously produced. The reaction mixture is quickly agitated, then maintained at room temperature for 30 minutes in the dark to complete the reaction. The reaction medium's absorbance is measured at 515 nm [10].

Antibacterial activity

The technique of diffusion on solid medium has been used [11]. It is a method similar to that of antibiogram which consists in determining the sensitivity of a bacterial strain to one or more products. A sterile disc of Whatman paper (n^o 1), the test items are submerged in a 6 mm diameter. (Pure extract, Extract diluted to 1/2,and Extract diluted to 1/4). The three soaked discs of the three concentrations are placed on agar which is previously inoculated with strains and an inoculum concentration between 106 and 108 CFU/ml. Petri dishes are incubated at 37°C for 18 to 24 hours. After incubation, if the product is toxic to the bacterial species, it forms a halo or zone around the disc. The diameter of the inhibition zones is measured in millimeter's (mm). Control discs (sterile distilled water) and comparison discs (Penicillin 10 mg) are included in the tests. Each test of the antibacterial activity of the extract is performed in triplicate.

3. RESULTS

Phytochemical qualitative analysis

To determine if chemical groups were present in the studied plant extract, phytochemical screening was performed (Table 0I), for Understand the active components responsible for biological actions.

Table 01: Phytochemical qualitative analysis of *Ephedra alata* aerial parts extract.

Chemical constituents	Aerial parts of *Ephedra alata*
Alkaloids	(+)
Flavonoids	(+)
Anthocyans	(+)
Saponins	(+)
Terpenoids	(+)
Leucoanthocyans	(+)
Tannins	(+)
Steroids	(-)
Cardinolids	(-)

(−) Absence of phytochemicals compounds. (+) Presence of phytochemicals compounds.

Determination of total phenolic content

The phenolic content obtained from aqueous extracts was estimated using a calibration curve and a reference extract (Gallic acid) at different concentrations. Results are expressed in mg Gallic acid equivalent per mg extract (mg GAE/mg extract). The calibration curve is established with a correlation y = 0.0009x + 0.001 and coefficient R2 = 0.9926. The results were represented in Table 2.

Table 02 : Quantitative analysis of total phenolic of *Ephedra alata* aerial parts extract.

	Aerial parts of *Ephedra alata*
Total phenolic (mg GAE/mg extract)	13.72 ± 0.02

DPPH free radical scavenging assay

Ephedra alata aerial parts extract was tested for antioxidant activity by using DPPH free radical scavenging assay. The results of antioxidant activity were summarized in figure 01, and DPPH free radical scavenging of plant extract was compared to that of the standard (Ascorbic acid).

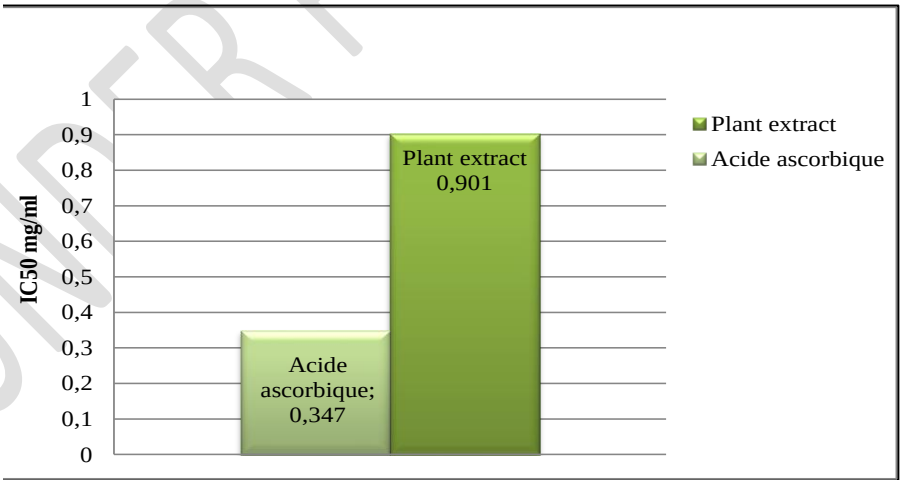

Figure 01: Antioxidant activity by using DPPH of *Ephedra alata* aerial parts extract.

Antibacterial activity

The results of the different tests performed with five strains of bacteria used for the products tested are grouped in Tables 3. The results show that there is a great heterogeneity in the results obtained, the antibacterial action is more or less important in accordance with the nature of the strain. Better resistant registries for both bacterial species: *Staphylococcus aureus* and *Proteus mirabilis*.

Table 03: Inhibition zones (mm) of Antibacterial activity for *Ephedra alata* aerial parts extract.

Strains	Tested products				
	Pure Ext	Ext diluted to 1/2	Ext diluted to 1/4	Water	Penicillin 10 mg
Escherichia coli	1.12 ±0.32	0.89 ±0.98	0.56 ±0.37	00 ±00	06.15 ±1.22
Pseudomounase aeruginosa	5.83 ±1.53	5.67 ±0.74	4.33 ±0.53	00 ±00	00 ±00
Staphylococcus aureus	00 ±00	00 ±00	00 ±00	00 ±00	10 ±0.83
Proteus mirabilis	00 ±00	00 ±00	00 ±00	00 ±00	05.03 ±1.22
Serratia fonticola	6.33 ±1.86	4.67 ±2.24	4.33 ±1.56	00 ±00	10 ±2.19

The natural products tested of *Ephedra alata* still manage to limit their development on ranges between 5.83 and 5.67 mm for *Pseudomonas aeruginosa*. This effect is often greater than that recorded in the presence of the antibiotic (Penicillin). *Staphylococcus aureus*, although considered as a highly resistant species to most antibiotics, it has been shown to be sensitive to the effect of Penicillin.

4. DISCUSSION

The phytochemical content varies depending on the product concerned. These levels may be associated with several factors such as growth and maturity conditions, genotype, storage conditions and extraction methods [12]. In this case, these factors resulted in a high level of plant extract (13%) for aerial parts of *Ephedra alata*. Moreover, previous studies show that methanol and water are the most widely used solvents for high recovery of phenolic compounds [13,14,15].

The results of the phytochemical analysis carried out on the aqueous extracts of the aerial part of *Ephedra alata* showed the presence of certain active compounds Tannins, Flavonoids, Anthocyans, Saponins, Alkaloids, Terpenoids and Leucoanthocyans when sterols and Cardinolids are absent, these results are compatible with the literate [16,17,18]

Phenolic compounds are considered important contributors to the biological activities of plants [19]. These compounds possess various biological activities such as Antibacterial [20]. Antifungal [21], Antidiabetic [22], Hepatoprotective [23], Haemato-protective [24]…For this reason, total phenolic content of *Ephedra alata* aerial parts were determined in this study. The results show that total phenolic content are abundant in the aerial part of *Ephedra alata* (13.72 ± 0.02 mg GAE/mg extract). This may be related to the harsh climatic conditions of the places where they grow (high temperature, high sun exposure, drought and salinity) that stimulate the biosynthesis of secondary metabolites such as polyphenols [25].

Antioxidant potential of polyphenols is great interest due to its chemo-protective against degenerative diseases such as neurological and cardiovascular diseases and its inhibitory effect of lipid peroxidation of foodstuffs [26]. Oxidative processes are multiple and the nature of antioxidant activity can be multifaceted and attributed to different mechanisms like the trapping of free radicals, chelation of transition metal ions, initiation prevention of EROs chain producing reactions and decomposition of peroxides. [27] Thus, the combination of several complementary antioxidant tests is useful in assessing the antioxidant potential of extracts [28]. The DPPH radical method used in this study. It's a common process for the antioxidant activity of the sample under investigation is estimated by the degree of discoloration of the DPPH solution [29].

Our results indicate that the aqueous extract has a high activity against scavenging assay of free radical DPPH with an IC_{50} =0.901 mg/ml. In a similar study on ethanol extracts from Ephedra procera

(same genus of our plant species) the authors reported an IC_{50} level of 0.056 mg/ml [30], a very low level, indicating an important antioxidant activity compared to ours, because the reductive power of a compound can serve as a significant indicator of its potential antioxidant activity, even that there is a direct correlation between antioxidant activities and the reduction power of the components of some plants [31,32]. In addition, aqueous extracts are assumed to contain polar compounds such as flavonoids and glycosides that may be responsible for their antioxidant activity [33].

Results found of antibacterial activity confirm once again the efficacy of the extracts of medicinal plants and their antiseptic power which rivals that of antibiotics [34, 35]. Numerous studies have demonstrated the antibacterial effect of natural active substances. Indeed, an article in the journal of life sciences [36]. reports that natural products from *Ephedra foliate* extracts has inhibitory and lethal effects on a variety of strains. For strains tested, only *Staphylococcus aureus* and *Proteus mirabilis* showed a high performance in resistance to the natural products tested and all concentrations used. The crude extract of many plants have also been shown in several investigations to have some antibacterial action. While these plants extracts and essential oils severely restrict the development of strains, [37,38].. According to Candan et *al* (2003) [37], hydrosoluble substances have a lower effect compared to no hydrosoluble substances. This probably refers to the ability of fat-soluble molecules for inserting into the bacterial membranes cells and damages them.

The constituents of the phenolic structure are very active against microorganisms and act as denaturants of proteins. These phenolic compounds are capable of binding to certain proteins and enzymes, thereby modifying the enzymatic equilibrium [39]. Finally, the activity of an extract is probably due to the presence of synergy between a number of components, which, when separated, become inactive individually [40].

5. CONCLUSION

Through the study of *Ephedra alata* phytochemical profile, antioxidant and antibacterial activity face pathogenic and multiresistant bacterial strains. It seems that these phytochemical compounds of *Ephedra alata* have an important antioxidant and antibacterial power on the tested germs responsible for infectious diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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