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Medicinal Plant Roots Microbiome in Antibiotic Resistant Fight Therapy†

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Abstract: In the framework of enhancing a wild and invasive Mediterranean plant which aerial parts are known for its traditional uses as natural insecticide, fire barrier, veterinary and human medicinal plant, in this perspective aerial and roots parts were investigated chemically and microbiologically investigated as the microbial world has caught immense attention in recent years for both humans and plants, it is recognized that microbes hold an enormous potential to increase host health. To achieve our hypothesis, quantification of the main secondary metabolites; total polyphenols and flavonoids, in addition to in vitro antibacterial and antifungal activities tested by discs diffusion method on agar medium, were carried out; the effectiveness of tested extracts has been demonstrated against five pathogen bacterial and fungal referential strains, then compared. Obtained results exhibit aerial part as better phenols sources, whereas roots extract showed better in vitro antimicrobial activity, which confirms that microbial resistance potential of roots is not attributed or correlated to phenols content. The present study open large perspectives to encourage intelligent culture and exploitation of such invasive plants and its bioactive compounds assessment, roots microbiome and antimicrobial mechanism, in order to develop low cost and safe biosanitary products.

Keywords: medicinal plant roots; phenols; antimicrobial activity

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1. Introduction

Medicinal plants are traditionally used since ancient times to treat common illnesses and more serious diseases. Their actions come from their chemical compounds: primary and secondary metabolites, and particularly from the synergy between the various compounds they contain [1].

In Algeria, several authors have published books on traditional phytotherapy and ethnobotany, however; this country remains poorly explored, even though it has considerable natural resources in different ecosystems and considerable floristic diversity. In this area, old knowledge and therapeutic practices are exclusively preserved [2]. Nevertheless, it is the role of modern researches to prove the efficiency of such practices.

In this regard, the present work aims at valorizing the medicinal plants of Algeria and possible discovery of metabolites responsible of therapeutic effect.

2. Experiments

2.1. Plant Materials

The plant used in our study is a wild medicinal plant that was harvested during fall, in Ali Mendjeli area, Constantine. Different parts of the plant were investigated to obtain four extracts namely:

- E1: Roots
- E2: Total aerial part
- E3: Flowers
- E4: Leaves and branches

All used chemicals are of analytical quality.

2.2. Extraction

After the plant is harvested perfectly cleaned and dried in dark place, each part of it is ground into a powder using a mortar and a pestle, then put in a sterile jar of shaded glass and filled with ethanol with a ratio of 1/3 (plant powder/solvent). The whole content is then stirred, tightly closed and left to macerate at room temperature for a month.

Each macerate is filtered using a Whatman paper N °4, and evaporated at 40 °C under reduced pressure, maintained with a vacuum pump, to give the crud ethanol extracts conserved aseptically in the freezer for future uses in the quantitative analysis.

Yields are calculated according to the following formula:

Yield% = (Crude extract mass/powder mass) * 100

2.3. Total Polyphenols Content

0.2 mL of each sample was firstly mixed with 1ml of diluted Folin–Ciocalteu reagent (5/10 H_2O) by vortexing. After that, 0.75 mL of Na_2CO_3 (7.5%) are added. Then, the reaction mixtures are further incubated for 2 h at room temperature in the dark, and finally, the absorbed optical density is recorded at the wavelength of 765 nm [3–4].

2.4. Total Flavonoid Content

0.4 mL of diluted sample with 1ml ethanol is separately mixed with 1 mL of 2% aluminum chloride methanol solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture is measured at 430 nm with spectrophotometer [5].

2.5. The Antimicrobial Activity Analysis

The antimicrobial susceptibility and resistance tests of our extracts were carried out according to the Agar disk-diffusion testing developed in 1940 [6].

Discs (Whatman No. 1, 6 mm diameter) are impregnated with each extract and then applied to the surface of the agar plates which have been seeded by spreading the microbial suspension. The seeding is carried out in such a way to ensure a homogeneous distribution of the bacteria. The petri dishes are incubated during 24 h at the appropriate temperature 37 °C in the laboratory oven, and the resulting inhibition zone diameter was measured in millimeters using a ruler.

Antimicrobial activity is determined in terms of the diameter of the inhibition zone produced around the discs.

3. Results and Discussion

3.1. Total Phenol and Flavonoid Compound Content Results

The total phenol content in (Figure 13) shows the highest concentration in polyphenols in extract E3 (flowers) with a value of (278.40 ± 0.01) DE; followed by a relatively low value of (144.29 ± 0.00), (138.30 ± 0.00) μg EGA/mg DE for E4 (stems and branches) and E2 (aerial part). As we can see in the figure; the extract that contains the lowest concentration equal to (76.23 ± 0.00) μg EGA/mg DE is E1 (roots).

Similarly to the phenol content, the extract E3 (flowers) contains the highest concentration of flavonoids with a value of (6.57 ± 0.00) μg QE/mg DE followed by E2 (aerial part) (34.57 ± 0.04) μg QE/mg DE, E1(roots) (27.53 ± 0.01)) μg QE/mg DE and finally E4 with (23.21 ± 0.00) μg QE/mg DE as the lot concentration.

3.2. Evaluation of Antimicrobial Activity

3.2.1. Antibacterial Test

The diameters results of the growth inhibition zones showed variation in the antimicrobial properties of the different parts of the plant and revealed that the inhibition zones for:

- Extract E1 (roots) are present only for the following strains: *Ecolab* with a highest diameter equal to (15.00 ± 0.50), followed by (12.00 ± 0.00) for *P. aeruginosa*. Meanwhile, the *K. pneumonia* and *S. aureus* did not show any zone of inhibition, which explains their resistance.
- Extract E2 (the aerial part) showed the inhibition zone just for *E. coli* with a diameter equal to (20.00 ± 0.50) while the inhibition for the three other stains was null.—Extract E3 (flowers), a zone of inhibition was observed only for *E. coli* with a diameter of 12.00 ± 0.00). The other strains showed a resistance toward this extract.
- In contrast, concerning the extract E4 (Stems and branches) no antibacterial activity was observed against the four strains.

  Gentamicin (10 μg/disc) and Nalidixic (30 μg/disc) were used as positive control.

3.2.2. Antifungal Activity Test

The diameters results of the growth inhibition zones showing antifungal activity against *Trichoderma harzianum Rifai* reveal that the zones of inhibition for the extract E1 (the roots) and the extract E2 (the aerial part) have the highest and the same diameter of (20.00) which is a strong activity, however the extract E3 (the flower) and the extract E4 (Stems and branches) showed a modest activity with a diameter equal to (10.00).

4. Conclusions

The present work aimed at promoting Algeria's medicinal plants in order to facilitate people's access to improved traditional medicines with less side effects and toxicity risks. In order to validate the traditional use of the wild plant species used in the present study, and look for alternatives to synthetic chemicals, this research has been conducted based on the quantitative determination of total polyphenols, total flavonoids and the assessment of antibacterial and antifungal properties of studied plant.

Through this study, aerial part was better phenols source, whereas roots extract exhibit better in vitro antimicrobial activity, which confirms that microbial resistance potential of roots is not attributed or correlated to phenols content.

The present study open large perspectives to encourage intelligent culture and exploitation of such invasive plants and its bioactive compounds assessment, roots microbiome and antimicrobial mechanism, in order to develop low cost and safe biosanitary products as fungicide and bactericide.

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