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Biological Assessment of a Mediterranean Invasive Weed for a Better Ecological Farming Management †

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Abstract: In the framework of enhancing wild medicinal plants of the Mediterranean flora, the present work investigates phytochemical screening of different parts' extracts of a wild medicinal plant from Asteraces family *Inula viscosa* L.: roots, leaves, flowers and aerial parts. It also highlights the quantification of the main secondary metabolites; total polyphenols and flavonoids and its correlation with in vitro antioxidant and antimicrobial activities. Biological tests have shown encouraging results for the antioxidant activities namely: reducing power FRAP test, hydrogen peroxide and hydroxyl radical scavenging, and exhibit flowers extract as promising source of phenols and potent antioxidants with the ability of breaking hydroxyl free radical chain generating, the main responsible of oxidative stress, on the other hand antibacterial and antifungal activities tested by discs diffusion method on agar medium, were carried out; and the effectiveness of tested extracts has been demonstrated against five pathogen bacterial and fungal referential strains. Obtained results exhibit aerial part as better phenols sources, whereas roots extract showed better in vitro antimicrobial activity. Obtained results showed nice correlation and open large perspectives on bioactive compounds assessment for the development of an ecological farming adjuvant.

Keywords: invasive weed; polyphenols; flavonoides; antioxidant activity; antimicrobial activity

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1. Introduction

Inula viscosa (L.) Aiton (Family Asteraceae, Tribe Inuleae), currently classified as *Dittrichia viscose* (L.) Greuter, is a woody, scented plant of Mediterranean distribution, that grows in sandy and pebbly stretches near rivers, and is also very common in abandoned lots, disturbed land, and edges of paths and roads, where it may be very abundant, even appearing as a monoculture. Its flowers spectacularly in September, showing numerous capitula of yellow flowers. In winter, the upper parts of the stem die, remaining dry a long time, and most of the leaves fall, so that the plant is considered semideciduous. Its wide use in folk medicine in the Mediterranean area, for its antipyretic, anti-inflammatory, antifungal, and anthelminthic properties [1].

Extracts of the species are particularly rich in terpenoids, in particular eudesmane acids, notably ilicic acid and α-costic acid. Flavonoids are also abundant, especially during flowering [2].

Some of these compounds are known for their biological activities against a wide range of micro- and macroorganisms. Scientific evidence reported that derivates of caffeic acids and flavonoids were the compounds mainly related to inhibition of bacterial and fungal growth, whereas sesquiterpene lactones and eudesmane sesquiterpenes were most active against nematodes, mites, insects and parasitic plants [3].

The species is very resistant to adverse abiotic conditions common in degraded environments like frequent cutting, fire, drought, and nutrient poor soil [4]. It is important as food for the caterpillars of certain butterflies, moths as well several parasitoids of economically important pests [5].

In parallel with the advances in research aimed to underline the potential of D. viscosa extract as medicaments for human care, the recognized antifungal activity attracts the attention of scientist working in agricultural sciences and food preservation. In a more general view, a wide branch of agricultural scientists focused their attention to natural products as surrogate of synthetic products for crop protection. (bioassay testing the effect on Oomycetes, Ascomycetes and Basidiomycetes, on grapes to control the Plasmopara viticola, causal agent of downy mildew, control a number of diseases caused by oomycetes and fungi in horticultural crops Alternaria solani …, [6].

Moreover, some extracts have an inhibitory effect on parasitic plants seeds. and against herbivorous insects and the nematode Meloidogyne javanica. expressing as antifeedant activity, reducing the Spodoptera littoralis larvae feeding and affecting the feeding behaviour of two aphids (Myzus persicae, and Rhopalosiphum padi) [7].

studies the effect of D. viscosa extracts against Varroa destructor, an external parasitic mite that attacks and damage the honey bees (*A. mellifera*): costic acid exhibited potent in vivo acaricidal activity against the parasite so the extract could be used as a safe, low-cost and efficient agent for controlling varroosis in honey bee colonies. The authors reported that the sesquiterpene lactones isolated from *D. viscosa*, namely inuloxins A, C, and D have statistically significant herbicidal activity against these economically important parasitic weeds.

Also, essential oil rich in short-chain volatile compounds with aldehydes, alcohols and esters functional groups as well as long chain fatty acids with esters and alkyls, shown to inhibit in vitro the growth of a wide range of opportunistic and pathogenic microorganisms.Biological activity was also tested in plant defence against phytopathogenic microorganisms along with macroorganisms like mites, insects and parasitic plants. In addition, extraction method, part of plant used, target organism, location and period of harvest are the key variables affecting the yield, composition and bioactivity of plant extracts [3].

In the present study, information about the phytochemical composition and the biological activity of *D. viscosa* against bacteria, fungi, and oxidative stress is given and its use is being viewed with interest in pharmaceutical, cosmetic, food and agricultural sectors.

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 1 mL 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 [8,9].

2.4. Total Flavonoid Content

0.4 mL of diluted sample with 1 mL 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 [10].

2.5. The Antioxidant Activity Analysis

In this study, three complementary tests were used to assess the antioxidant activity: the ferric reducing antioxidant power (FRAP), the hydrogen peroxide activity and the hydroxyl radical scavenging assays

2.5.1. The FRAP Assay

The ferric reducing capacity of extracts was investigated by using the potassiumferricyanide-ferric chloride method [11]. Briefly, 0.1 mL of each of the extracts at different concentrations, 0.4 mL of phosphate buffer (pH 6.6), and 0.5 mL of potassiumferricyanide $K_3Fe(CN)_6$ (1%) are mixed and incubated at 50 °C for 20 min, to reduce ferricyanide into ferrocyanide. The reaction is stopped by adding 0.5 mL of 10% (*w*/*v*) trichloroacetic acid. Finally, it is mixed with 0.4 mL of distilled water and 0.1 mL of $FeCl_3$ (0.1%) and the absorbance is measured at 700 nm.

2.5.2. Hydrogen Peroxide Scavenging Assay

The ability of the plant extracts to scavenge hydrogen peroxide is estimated according to the method reported by [12] with minor modifications. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (1 M pH 7.4) (0.63 mL H_2O_2 + 199.4 mL phosphate buffer pH 7.4). 1 mL of each sample of the concentration range is added to 0.1 mL of hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm is determined after 10 min of incubation. Ascorbic acid is used as a standard.

2.5.3. Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity of the extracts is determined according to the salicylic acid method reported by [13].

The reaction mixture contained 0.4 mL of each extract and concentration, 0.24 mL iron sulfate $FESO_4$ (111.2 mg/10 H_2O), 0.2 mL H_2O_2 (0.3 µL H_2O_2/199.7 µL H_2O), 0.8 mL acid salicylic (4 mg/10 mL H_2O), then it was incubated at 37° for 30 min in a water bath, after that 0.36 mL H_2O was added. The absorbance of the mixtures is measured at 510 nm using a (UV/Vis) spectrophotometer. The ascorbic acid is used as the positive control compound.

2.6. 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 [14]

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 Biological Activities

3.2.1. Antioxidant Activity

- **Reducing Power Test Results**

Flower extract (E3) has the most interesting potential among the other extracts with the lowest A0.5 of the order of 0.037 μg/mL. For the roots (E1), the aerial part (E2) and the stems and branches (E4) extracts, they respectively present less potential with values of 1.56, 2.7 and 3.15 μg/mL. All of them presenting a much better activity than the standard which has an A0.5 equal to 10.2 μg/mL.

According to the 4 curves, the ferric reducing power of the four extracts of our medicinal plant showed a high correlation with the phenol content with a correlation that varies form (0.904–0.9902). This explains the fact that the ferric reducing power increases with the increasing of the phenol content and gives an idea about the contribution of the phenol compounds in the antioxidant activity of the different parts of our medicinal plant.

- **Hydroxyl radical scavenging assay results**

For the Hydroxyl radical scavenging assay; the extract of the aerial part E2 showed the lowest A0.5 (184.28 μg/mL) and the closest to the one of the ascorbic acid (1.69 μg/mL). This gives it the highest antioxidant potential among the other extracts that express higher values of A0.5, and thus have a lower antioxidant potential as followed: The stems and branches extracts E4 with (649.46 μg/mL) followed by the flowers ones E3 (829.22 μg/mL) and then the roots' E1 with a very high A0.5 of more than 1000 μg/mL. This makes it the extract with the lowest antioxidant potential. The hydroxyl radical scavenging results showed a very high correlation with the total phenol compounds content in the four extracts, with a correlation coefficient that varies form (0.9522–0.9979). This indicates the correlation between the two parameters and shows the obvious contribution of the phenol compounds in the antioxidant potential observed in the different extracts from the medicinal plant studied.

- **The hydrogen peroxide activity assay**

The stems and branches extract (E4) has the most interesting potential among the other extracts with the lowest IC 50 of the order of 4.73 μg/mL which is the closest to the one of the ascorbic acid 2.56 μg/mL. For the roots (E1), the aerial part(E2) and the flowers (E3) extracts respectively present less potential with values of 5.13 and 5.59 μg/mL (same IC 50 for (E3) and (E4)).

According to the 4 curves, the hydrogen peroxide activity of the four extracts of our medicinal plant showed a low correlation with the phenol content with a coefficient of correlation that varies form (0.4356–0.5215).

3.2.2. Antimicrobial Activity Results

- **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.

- **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 a Mediterranean invasive weed as a sustainable Biosourced therapeutic agent for human health treating as well as farming management and look for alternatives to harmful synthetic chemicals, this research has been conducted based on the quantitative determination of total polyphenols, total flavonoids and the assessment of antioxidant, antimicrobial and antifungal properties of studied plant.

Through this study, a correlation between the total phenol content, the flavonoid content and the antioxidant potential was establish and which turned out to be probably due to the plant richness in phenol compounds, flavonoids and other secondary metabolites, making it a promising source for treatment of many human and plant diseases by using it as multidisciplinary natural phytosanitary agent.

Author Contributions: M.T.N. conceived and designed the experiments, analyzed the data and wrote the paper; Z.A. and Y.A. performed the experiments; Z.K. contributed reagents/materials/analysis tools. All authors have read and agreed to the published version of the manuscript.

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