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Comparative Analysis of a Mediterranean Wild Medicinal Plant Parts' *In Vitro* Biological Activities †

Toma Nardjes Mouas [1,*], Zahia Kabouche [1], Zeyneb Aissani [2] and Khadidja Yasmine Ariane [2]

[1] Université frères Mentouri-Constantine 1, Laboratoire d'Obtention de Substances Thérapeutiques LOST, Campus Chasbet Ersas, 25000 Constantine, Algeria; Email
[2] 25000 Constantine, Algeria; Email (Z.A.); Email (K.Y.A.)
* Correspondence: mouas.toma.nardjes@umc.edu.dz
† Presented at the 1st International Electronic Conference on Biological Diversity, Ecology and Evolution, 15–31 March 2021; Available online: https://bdee2021.sciforum.net/.

Abstract: In the framework of enhancing medicinal plants of the Mediterranean flora, the present work investigates phytochemical screening of different parts' extracts of a wild medicinal plant from Asteraces family: 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, 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, SAR studies and clinical trials.

Keywords: Asteraces; polyphenol; Flavonoides; antioxidant activity; antimicrobial activity

Citation: Mouas, T.N.; Kabouche, Z.; Aissani, Z.; Ariane, K.Y. Comparative Analysis of a Mediterranean Wild Medicinal Plant Parts' In Vitro Biological Activities. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

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 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 [5].

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 [6]. 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 [7] 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 [8].

The reaction mixture contained 0.4 mL of each extract and concentration, 0.24 mL iron sulfate FESO4 (111.2 mg/10 H_2O), 0.2 mL H_2O_2 (0.3µL 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 [09]

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 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 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 diseases by using it to synthesize new phytomedicines.

Author Contributions: M.T.N. conceived and designed the experiments, analyzed the data and wrote the paper; Z.A. and K.Y.A. performed the experiments; Z.K. contributed reagents/materials/analysis tools.

Acknowledgments: Authors would like to thank Algerian Ministry of Higher Education and Scientific Research DGEFS, and the Algerian Directorate General for Scientific Research and Technological Development DGRSDT for financial fund.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Ensa, R.L. Using medicinal plants in Algeria. American Journal of Food and Nutrition **2011**, doi:10.5251/ajfn.2011.1.3.126.127.
2. Ouelbani, R.; Bensari, S.; Mouas, T.N.; Khelifi, D. Ethnobotanical investigations on plants used in folk medicine in the regions of Constantine and Mila (North-East of Algeria). *J. Ethnopharmacol.* **2016**, *194* doi:10.1016/j.jep.2016.08.016.
3. Singleton, V.L.; Rossi, J.A.J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* **1965**, *16*, 144-158.
4. Müller, L.; Gnoyke, S.; Popken, A.M.V.; Böhm, V. Antioxidant capacity and related parameters of different fruit formulations. *LWT Food Sci. Technol.* **2010**, *43*, 992–999.
5. Topçu, G.; Ay, A.; Bilici, A.; Sarıkürkcü; C; Öztürk, M.; Ulubelen, A. A new flavone from antioxidant extracts of *Pistacia terebinthus*. *Food Chem.* **2007**, *103*, 816–822.
6. Oyaizu, M. Studies on products of browning reactions: Antioxidative activities of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **1986**, *44*, 307–315.
7. Ruch, R.J.; Cheng, S.J.; Klaunig, J.E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea. *Carcinogenesis* **1989**, *10*, 1003–1008, doi:10.1093/carcin/10.6.1003.
8. Smirnoff, N.; Cumbes, Q.J. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* **1989**, *28*, 1057–1060, doi:10.1016/0031-9422(89)80182-7.
9. Heatley, N.G. A method for the assay of penicillin. *Biochem. J.* **1944**, *38*, 61–65, doi:10.1042/bj0380061.