Valorization of essential oils, hydrosols and extracts of Algerian Inulaviscosa leaves

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Abstract

This study focuses on the valorization of the extracts of Inulaviscosa leaves in terms of yield and antioxidant properties. The recovery tests of Inulaviscosa essential oils using three methods (Steam distillation, Hydrodistillation, Hydrodiffusion) showed that the essential oils even when their yields are low, presented very valuable antiradical activity. The hydrosols obtained from these three processes revealed a great presence of phenolic compounds with important antiradical activity. The extraction tests on Inulaviscosa leaves performed in a Soxhlet showed decreasing yields depending on the polarity of the solvent used. The same observation stands for the antiradical activity. In all cases, the use of water, the unique green solvent with low cost, gave the best results in terms of polyphenols yield and antiradical activity.
This study highlighted the influence of the nature of the process and the solvent used on yield and antiradical power of the recovered extract. These results are very promising as the antioxidant phenolic compounds recovered using an environmentally-friendly cleaning process constitute a good source of natural antioxidants for food, cosmetic and pharmaceutical industries.

Keywords: *Antiradical activity, essential oils, extraction, Inulaviscosa, polyphenols content.*

I. Introduction

In recent years, aromatic and medicinal plants have attracted great interest in different areas. The *Inulaviscosa*, known locally as "*Amagraman*", is a perennial herbaceous plant distributed throughout the Mediterranean; it was widely used in traditional medicine. Indeed, this plant has been of great importance in the biological field quite a long time, its oils were used for their antipyretic, anti- inflammatory, antiseptic effects, its antiphlogistics activities and in the treatment of diabetes [1, 6]. It is recently known for its antioxidant properties [7]. Comparing it to other plants, few studies and scientific research were interested in this plant. Its essential oil [8-14] and its extracts [9, 12, 14-22] have been subject for several scientific researches. However its hydrosol was so far very little studied [8, 23].

The present work concerns the antioxidant activity of essential oils obtained by different methods: steam

distillation (SD), hydrodistillation (HD) and hydrodiffusion (Hdiff), of hydrosols and extracts recovered using different solvents. Fig. 1 represents the procedure followed.

II. Materials and methods

A. Plant material

The inulaviscosa was collected in April 2015 in El-Harrach 12 km east of Algiers. Some tests were performed with the fresh plant and the other with the dried one. The leaves were dried in the shade, in the dark, at a constant temperature of 18 ± 1 °C and used without any preliminary treatment.

B. Analytical methods

The total content of polyphenols (TPC) was determined by the Folin-Ciocalteu method [24], and expressed as milligram Gallic acid equivalent per g dry weight material (mg GAE.g DW^{-1}).

The antioxidant activity (AA) was determined using the DPPH test [25]. The antiradical activity was calculated from the formula:

AA (%) = (Abs (blank) - Abs (sample)) / (Abs (blank))

AA: antiradical activity in %.

Abs (blank): absorbance of DPPH dissolved in methanol.

Abs (sample): absorbance of the sample (essential oil, hydrosol or extract) in methanol.

All measurements were performed three times, the values of antioxidant activity (IC_{50}, AA %) and total polyphenol were expressed as average value ± standard deviation.

C. Extraction processes

1) Steam distillation (SD): The tests were carried out by placing a known mass of leaves (8 to 20 g), having a pre-determined humidity (19.5 to 79.5%), in a thermally insulated column. During 5 hours, steam feeds the column at the bottom with an average flow (4.5 to 18.4 $mL.min^{-1}$) with a cohobation system.

2) Hydrodistillation (HD): The tests were carried out by immersing a known mass of leaves (23.7 to 36.7 g) with a pre-determined humidity (21.7 to 74%) in a volume of boiling water, with an average flow (12 to 18.1 $mL.min^{-1}$). The tests were conducted with a cohobation system during 5 hours.

3) Hydrodiffusion (Hdiff): The tests were carried out by placing a known mass of leaves (10.39 g), with pre-measured humidity (21.7%), in a thermally insulated column. The steam supplies the column from the top with an average flow of 10.2 $mL.min^{-1}$ during 5 hours.

4) Extraction by volatile solvents: The extraction tests were carried out in a soxhlet with solvents of different polarities (400 mL): methanol, ethanol, acetone, ethyl acetate and hexane. A mass of 8 g was used for each test, the humidity was measured (13-17%) and extraction lasted 8 hours.

III. Results and discussion

A. Essential oils

Although their yields are sometimes very low (0.44 to 2.15% for SD, 0.09 to 0.62% for HD and 0.28% for Hdiff.), the antioxidant activity of the essential oils (diluted in 5 mL of methanol), varies from 35% to 87% for SD, 20% to 30% for HD and reaches 90% for Hdiff, depending on the condition of the plant and its humidity.

The antioxidant activity of essential oils obtained by hydrodiffusion is significantly higher than that of steam distillation and hydrodistillation. Indeed, for a concentration of 260 $\mu g.mL^{-1}$, the inhibition rate varies from 5 to 11% for HD, 10 to 17% for the SD and 92% for Hdiff.

The hydrodiffusion seems interesting in terms of antioxidant activity especially as it may be improved by looking for the optimal operating conditions. In fact, the flow rate is somewhat low, compared to the other two processes.

B. Hydrosols

The antioxidant activity of the hydrosols recovered during the extraction of the essential oils, for each of the processes, and the polyphenol contents are given in Tab. 1.

Examination of Tab. 1 shows that the inulaviscosa hydrosols are rich in polyphenols. This finding has already been made for the hydrosols of the inulaviscosa of Portugal reported by [8], which found a polyphenol content of 14.3 ± 0.6 mg $GAE.mL^{-1}$. We find that the hydrodistillation gives higher polyphenol contents, this can be explained by the fact that the leaves are in direct

contact with water allowing a good solubilization of polyphenols.

The antioxidant activity of hydrosols can reach 80% for HD for a concentration higher than 20 µg GAE.mL^{-1}, 85% for SD at a concentration higher than 1 µg GAE.mL^{-1} and 80% for Hdiff at a concentration above 2 µg GAE.mL^{-1}, as shown in Figs. 2 to 4.

This indicates the strong antioxidant power of the hydrosols we can associate with the high polyphenol contents. The IC_{50} of the hydrosols confirm that they possess high antioxidant activities. Compared to inulaviscosa of Portugal, Algerian inulaviscosa is better in terms of antioxidant activity, in fact the IC_{50} obtained in the study [8] is 4.0 ± 0.0 µg.mL^{-1}.

C. Extracts recovered in a Soxlet

The results of IC_{50} and polyphenol contents are summarized in Tab. 2.

The results of the DPPH test show that the methanolic extract has the highest antioxidant activity; Hexane showed the lowest antioxidant activity. We note that the polyphenol contents follow the same variation as the antioxidant activities, the methanolic extract is very rich in polyphenols, a linear variation is observed for the polar solvents (Fig. 5).

It has been reported that the majority of polyphenols are classified as hydrophilic antioxidants [26], [27]. Methanol was the most effective solvent for the extraction of antioxidant compounds, especially polyphenols. This may be due to the better solvation of the antioxidants following the interactions (hydrogen bonds) between the polar sites of the antioxidants and the methanol.

Ethanol is less effective than methanol in extracting antioxidants although their polarities are similar. This may be due to lower solvation, probably because of the ethyl radical, which is longer than the methyl present in the methanol.

Ethyl acetate yields polyphenol contents and antioxidant activities comparable to those of ethanol and higher than those of acetone. It is therefore deduced that the polyphenols of inulaviscosa are very soluble in ethyl acetate, which confers to its extracts an important antioxidant activity.

The acetone extract shows a relatively low antioxidant activity and polyphenol content, indicating that the solvent power of acetone is less effective, this can be explained by the fact that acetone is a proton receptor, while the other solvents are also donors.

Since hexane is an apolar solvent, a small amount of polyphenols has been extracted, therefore the antiradical activity of its extract is the lowest.

We deduce, from these results, that the antioxidant activity correlates positively with the polyphenol content and even the yields. The IC_{50} values found are comparable to those reported in Tab. 3. The polyphenol content of the methanol extract is greater than that obtained by [22] and that of the extract with the acetate is lower than that reported by [23].

IV. Conclusion

The recovered essential oils obtained by SD or HD showed an average antioxidant activity compared to the other asteraceae plants, whereas that recovered by hydrodiffusion has a higher antioxidant activity, reaching a 90% inhibition rate at a concentration of 260 µg.mL^{-1}. The hydrosols of the three processes are rich in polyphenols; their antioxidant activity is a function of the polyphenol content. The extracts recovered by the polar volatile solvents also exhibit good antioxidant activity, which varies linearly with the content of polyphenols. The best solvent is methanol. The best results have been obtained with hydrosols.

V. Figures

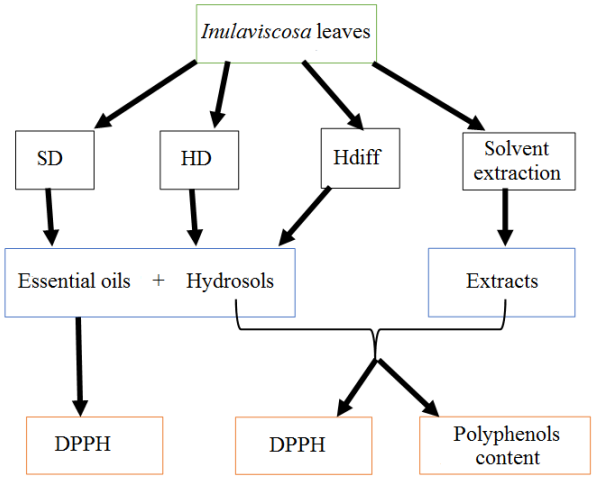

Figure 1. The experiments carried out in the present study

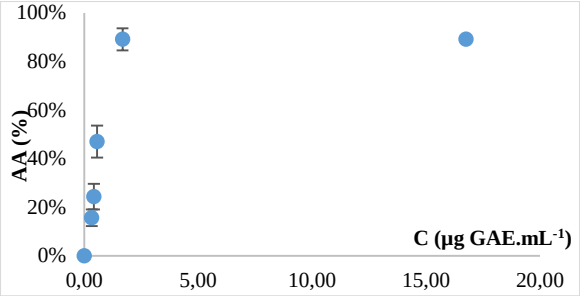

Figure 2. Antioxidant activity (%) of the hydrosols obtained by hydrodistillation as a function of the concentration of polyphenols

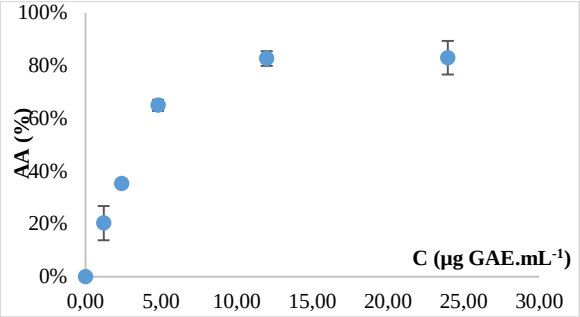

Figure 3. Antioxidant activity (%) of the hydrosols obtained by steam distillation as a function of the concentration of polyphenols

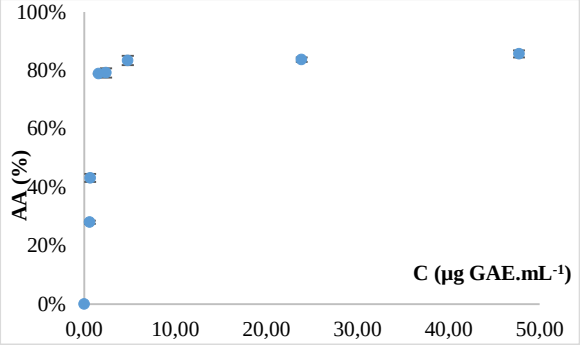

Figure 4. Antioxidant activity (%) of the hydrosols obtained by hydrodiffusion as a function of the concentration of polyphenols

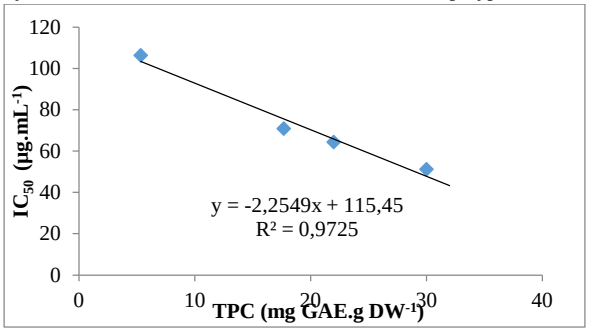

Figure 5. Variation of the IC_{50} with the polyphenol content of the different polar solvents used

VI. Figure legends

"**Figure 1.** The experiments carried out in the present study"
"**Figure 2**. Antioxidant activity (%) of the hydrosols obtained by hydrodistillation as a function of the concentration of polyphenols"
"**Figure 3**. Antioxidant activity (%) of the hydrosols obtained by steam distillation as a function of the concentration of polyphenols"
"**Figure 4**. Antioxidant activity (%) of the hydrosols obtained by hydrodiffusion as a function of the concentration of polyphenols"
"**Figure 5**. Variation of the IC_{50} with the polyphenol content of the different polar solvents used"

VII. Tables with heading

Table 1. Polyphenol contents and IC_{50} of inulaviscosa hydrosols

Process	TPC (mg GAE.g DW^{-1})	TPT (mg $GAE.mL^{-1}$)	IC_{50} (µg $GAE.mL^{-1}$)
HD	388,00 ± 40,56	1,64 ±0,17	4,50 ± 0,50
SD	291,02 ± 33,42	0,84 ±0,10	0,33 ±0,11
Hdiff	314,71 ± 92,29	1,55 ±0,46	0,90 ±0,10

Table 2. Antioxidant activities of inulaviscosa extracts represented by IC_{50} and polyphenol contents

Extract	IC_{50} (µg.mL^{-1})	TPC (mg GAE.g DW^{-1})
Methanol	5,33 ± 0,58	106,34 ± 1,49
Ethanol	17,67 ± 2,08	70,79 ± 1,20
Ethyl acetate	22,00 ± 2,00	64,32 ± 1,28
Acetone	30,00 ± 5,00	51,25 ± 0,71
Hexane	790,00 ± 10,00	17,03 ± 0,43

Table 3. IC_{50} values and total polyphenol contents found in previous work

Solvent	IC_{50} (µg.mL^{-1})	TPC (mg GAE.g DW^{-1})	reference
Methanol	4,27	62,61	[22]
Ethanol	18	84.85±1.38	[23]
Ethyl Acetate	28	103.71±2.78	

VIII. Acknowledgment

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IX. List of symbols

SD: steam distillation
HD: hydrodistillation
Hdiff: hydrodiffusion
AA: antioxidant activity
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
GAE: Gallic acid equivalent

IC_{50}: inhibitory concentration, which corresponds to a 50% reduction of the activity of DPPH in the reaction medium

TPC: total polyphenol content

X. References

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