

1. INTRODUCTION

Aromatic and medicinal plants essential oils have shown to be of a great interest for several areas such as pharmaceuticals, cosmetics and food fields. This has encouraged the development of oil extraction processes for the production of valuable natural substances used as natural active ingredients, hence banning synthetic ones and avoiding their harmful effects on the human health and the environment.

At this stage classical processes for the extraction of essential oil from natural plants are considered prior to the study of a non conventional extraction technique based on the supercritical fluids technology .

2. OBJECTIVES

- A priori and for comparison purposes, the extraction of oil from a local natural plant was performed by means of classical processes like Soxhlet extraction.
- In order to show in which form the plant (crude or powder) may lead to the best oil extraction yield with an adequate composition to achieve targeted biological activities.

3. MATERIAL AND METHODS

1. The natural plant :

The fir tree (*Abies numidica*) collected from the current harvest in Djebel El Ouach in Constantine, Algeria was washed, open air dried for a sufficient time and the needles were removed from the tree giving the raw material where an amount of which was ground.

2. Chemical reagents used :

Ethanol, Aluminum trichloride (AlCl₃), 95% purity Quercetin and sodium carbonate (Na₂CO₃); Gallic acid and Folin-Ciocalteau; vanillin and DPPH were provided by the usual suppliers.

3. Method :

The Soxhlet extraction was carried out using Ethanol as the least toxic solvent. The well known standard protocols were followed during the tests to determine the different considered biological activities.



4. RESULTS AND DISCUSSION

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1. Soxhlet Extraction of essential oil from fir :

The essential oil extractions from fir under the two considered forms, raw and powder, were carried out at the same operating conditions of time, temperature (ambient) and solvent. The obtained results are shown as follows: Table 1. Oil extraction yield for the raw and powder forms The oil extraction yield was higher for the powder form, due to a much greater mass transfer area, comparatively to the raw form.

Form	Raw	Powder
Extraction yield (%)	12.67	33.97
Mother concentration (mg/ml)	39.013	64.2

4.2 Antioxidant activity by DPPH test :

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical was used and after 30 min of reaction with fir oil, the absorbance was measured with a spectrophotometer (UVvis). The measurement was taken at 517 nm.

The percentage of inhibition was calculated according to the following equation:

$$\% \text{ of inhibition} = \frac{\text{Abs control positif} - \text{Abs sample}}{\text{Abs control positif}} * 100$$

Figures 1a & b show the inhibition curves for each form and express the inhibition percentage versus the oil concentration, hence the determination of the IC₅₀.

The obtained results showed that fir was rich in antioxidant compounds (polyphenols) that have radical activity. This activity varied depending on the form of the plant where the corresponding IC₅₀ were 70 and 30.5 µg/ml for raw and powdered fir, respectively. However an usual behavior was observed for the two forms for the concentration of 1000 µg/ml with a decrease of the % Inhibition while a 'plateau' was expected. An explanation to this behavior was not readily available but further explorations might be necessary at refined oil concentration step change.

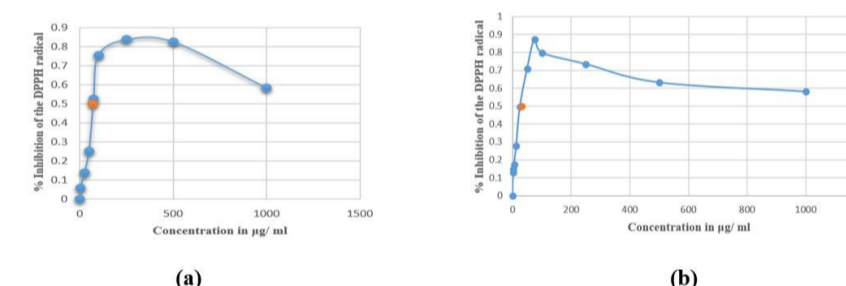


Figure. 1] Inhibition percentage versus the oil concentration a) Raw fir; b) Fir powder.

CONCLUSION

From this study it can be concluded that the fir essential oil had a very interesting antioxidant activity at low concentration, with the powdered form being more effective, probably due to the increase of the transfer surface area in the ground state between the plant and the solvent, enhancing the extraction of a considerable amount of antioxidant compounds. Regarding the types of polyphenolic compounds, the most dominant in the oil were phenolic acids compared to tannins and flavonoids.

All the obtained results will be exploited to assess the capabilities of the supercritical fluid extraction compared to the classical Soxhlet extraction, regarding the extraction yield and also the quality of the extracted oil and its biological activities.

REFERECES

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4.3The polyphenolic compounds test :

Polyphenolic compounds are secondary metabolites of natural plants. There are several types of polyphenol and each type has a special standard.

4.3aFlavonoids :

Flavonoids are phenolic compounds that are much considered since they are active ingredients in many plants or plant extracts.

The flavonoid calibration curve was plotted in a range of quercetin concentrations between 0 and 25 µg/ml.

The obtained results that were carried out at the same conditions of concentration showed that the considered plant had important flavonoids content, with the TFC of the raw form lower than that of the powder.

TFC (raw form) =28.44 mg EQ/gE

TFC (powder form) =38.7714 mg EQ/gE

It was observed that the absorbance of the solution increased with the increase of the flavonoid content, indicating that the yellow coloration produced increased proportionally to the quantity of flavonoids present in the extract.

4.3b Tannins:

The determination of condensed tannin contents was carried out by the acidified vanillin method. The results were determined from the linear regression equation of the calibration curve using Catechin as standard. The essential oil of fir contains a small quantity of tannin with that the content of condensed tannins in the crude form was higher than that of powder:

TTC (Crude form) = 0.8654 mg CE/gE

TTC (The powder form) = 0.781 mg CE/gE

4.3c Phenolic acids:

For this test, Gallic acid was used as a standard with the objective of plotting the calibration curve. The latter allowed us to determine the Equivalent Concentration which represents the content of Gallic acid in the essential oil composition. The total phenolic content (TPC) was determined by the Folin-Ciocalteau method.

It was noticed that phenolic acids were present with very high contents in the essential oil of fir compared to other polyphenolic compounds. They were also more present in the crude form than in the powder one (TPC (raw form) =132 mg GAE/g E, TPC (powder form) = 96 mg GAE/g E).

