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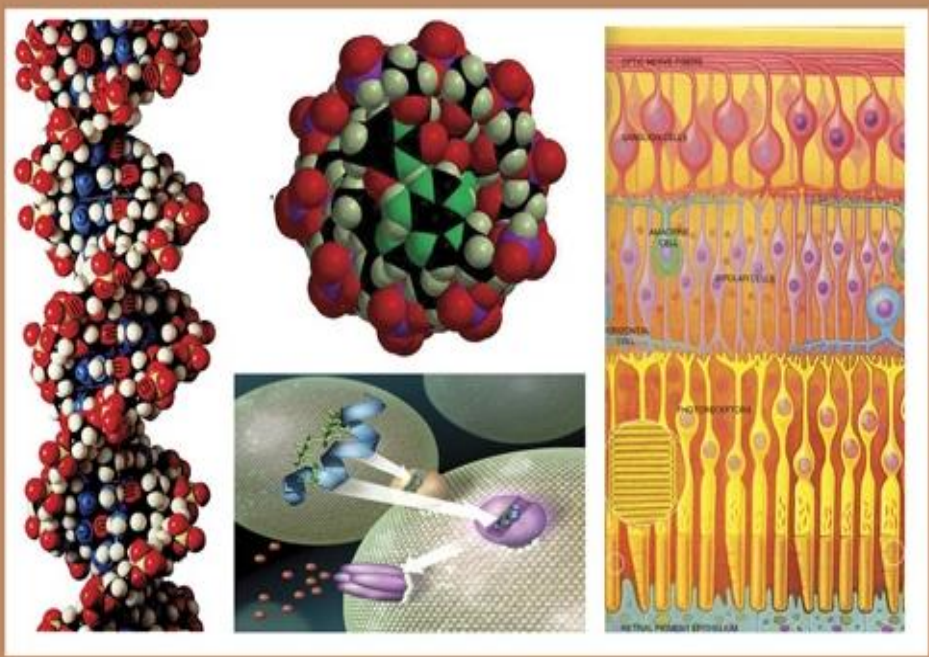

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Ethnobotanical and Phytochemical Study of *Inula viscose* L of the Western of Algeria

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ABSTRACT

Inula viscosa is a medicinal plant belonging to the Asteraceae famil**y.** This species is known under the name of Magramen. It is widespread in the Mediterranean region. An ethnobotanical survey was carried out in the city of Sidi Bel Abbes where this plant abounds. On the other hand, a phytochemical study of *Inula viscosa* is carried out. The determination of total phenols was carried out by using the Folin-Ciocalteu reagent, the quantification of flavonoids by the method with aluminum trichloride and sodium hydroxide, and that of the tannins condensed by the vanillin method under acidic conditions. Antioxidant activity was assessed using the free radical reduction method (DPPH). Thus, a toxicity test was carried out according to line 420 of the OECD. The results showed that this plant is involved in: the treatment of osteoarticular or rheumatic diseases (33%), dermatological infections (33%), nervous system (11%), digestive disorders (11%), and cardiovascular diseases (6%). The phytochemical screening allowed us to highlight the presence of secondary metabolites in the leaves of our plant; such as flavonoids, terpenoids, tannins, glycosides, and carotenoids. The content of the leaves in total phenols (31.46 mg AGE / g) is higher than that of the twigs (30.28 mg GAE / g). The amount of flavonoids is slightly higher in the leaves (133.93 mg EC / g) than in the twigs (37.12 mg EC / g). The prepared extracts have very significant anti-free radical activities since they recorded very remarkable IC50 values of 0.1 to 4.1 mg/ml. *Inula viscosa* is non-toxic for a dose of 2000 mg/ml or less.

INTRODUCTION

Aromatic plants represent an economic interest in pharmaceutical, agri-food, cosmetics, and pharmacy industries (Daira et *al*., 2016). These plants contain potential compounds. They attributed to the secondary metabolites that have the advantage of being of a great diversity of chemical structure. They possess a wide range of biological activities. In fact, the World Health Organization (WHO) estimates that about 80% of humanity uses traditional herbal preparations as primary health care (El Hilah et *al*., 2016).

Ethnomedicine is considered a set of empirical practices rooted in the knowledge of a social group.

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It is often passed orally from generation to generation to solve health problems. An alternative to Western medicine, it is strongly linked to the religious beliefs and practices of indigenous cultures. Knowledge of medicinal plants or phytotherapy is a major component of traditional medicine (Bussmann et *al*., 2002). The reasons for using traditional medicines include; the cultural acceptability of healers, the presence of local pharmacopeias, and relatively low-cost traditional medicine (Fournier et *al*., 1947).

Inula viscosa is known from a very long time ago. It was used during the Middle Ages and still for its various medicinal properties (Hunde et *al*., 2011). In the respiratory system, it acts as a sedative for coughs and bronchial spasms. It is a definite antiseptic for the respiratory tree (Benayache et *al*., 1991). As with all aromatic herbs, Viscous Inule corrects stomach and intestinal weakness, improves appetite, and is anti-emetic. In North Africa and the Mediterranean rim, *Inula viscosa* is known for its anthelmintic properties. Therefore, it occupies an appreciable place in traditional medications (Roulier et *al*., 1990).

This paper is focused on an ethnobotanical survey of *Inula viscosa* uses in the region of Sidi bel Abbes. The other part of this work is carried out on a phytochemical screening than the determination of total phenols, total flavonoids, and condensing tannin using spectrophotometer assay with antioxidant activity investigation.

MATERIALS AND METHODS

The Study Area:

Our study focused on the Sidi Bel Abbes region, located in the northwest of Algeria. Geographically, Sidi Bel Abbes has a strategic central position where it extends over approximately 15% of the territory of the north-western region of the country. 9150.63 km² is considered a relay due to its privileged location insofar as it is crossed by the main roads of this part of the country. The climate of Sidi Bel Abbes is characterised by low rainfall, rarely exceeding 300 mm / year. Only (20%) of the basin is covered with forests, mainly in the mountain ranges between El Haçaïba and Mouley Slissen as well as in the non-cultivable hilly areas which consist mainly of Aleppo pines and holm oaks (Bennabi *et al*., 2012).

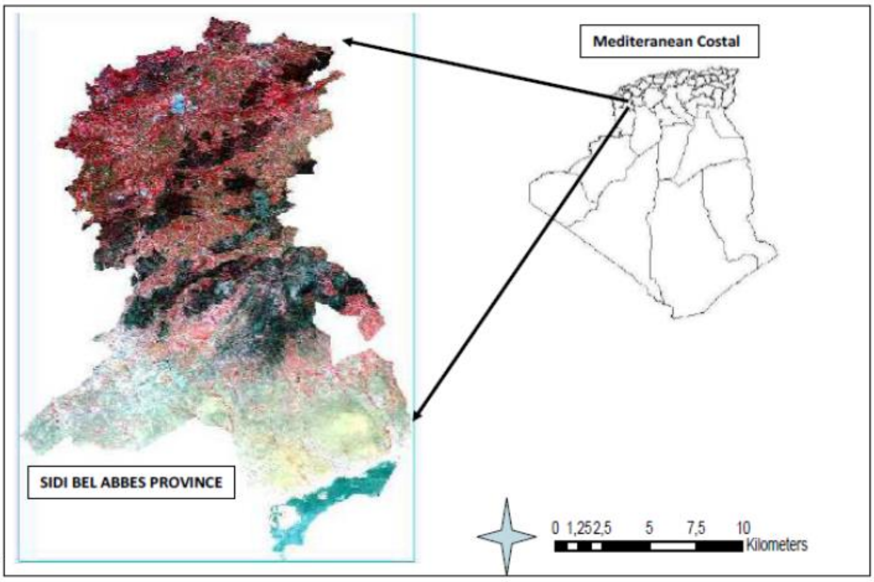

Fig. 1: Delimitation of de the study area.

Ethnobotanical Study:

An ethnobotanical study on the current status of *Inula viscosa* was carried out in the Sidi Bel Abbes region using 200 Surveys. To obtain more information on plant uses, during each interview abbés (Chief Town, Ben Badis, Sfisef, Sidi Lahcene, and Ténira). We have collected all the information on the species studied, including sex, academic level, age, the part used, the method of preparation, and diseases treated. In fact, individuals from the population are chosen randomly to express themselves freely even if they give details that are not requested.

Phytochemical Study:

A sampling of *Inula viscosa:*

The leaves and stems of *Inula viscosa* were harvested in January in the Sidi-Bel-Abbes region during the flowering period. The leaf and stems samples are cleaned, left to dry at temperature room in the shade with air, then grounded for extraction. The extraction was carried out by maceration of the vegetable powder (10 g) with three solvents: hexane, methanol, and ethanol for 24 hours. Then a fourth solvent (water) was used under reflux extraction.

Phytochemical Screening:

The detection of these chemical compounds is based on precipitation reactions, colour change, or examination under ultraviolet light. The phytochemical tests were carried out on the hydroalcoholic extracts by qualitative characterization techniques. The results obtained were evaluated as follows: +++: Strongly positive; + +: Moderately positive; +: Weakly positive; -: Negative

a.Flavonoids test: 1ml of the extract is treated with a drop of soda (NaOH). Intense in the presence of flavonoid (Daira *et al*., 2016).

b.Terpenoids test: we Take 2.5ml of each extract in test tubes and add 1ml of pure chloroform and 1.5ml of sulfuric acid (dropwise). A positive test is revealed by the appearance of a reddish-brown color[9].

c.Tannin test: We add 1ml of the extract, 2ml of distilled water, and 2 to 3 drops of Iron Trichloride ($Fe Cl_3$). A positive test is revealed by the appearance of a blue-black colour (presence of catechetical tannins). Green or blue-green and a precipitate: Gallic or elagic[10].

d.Phenol test: We treat 1ml of the extract with a few drops of nitric acid diluted to 1%.

The presence of phenols is evidenced by the appearance of an orange-yellow color (Trease E. et Evans W.C., 1987).

e.Tests for cardiac glycosides: we Take 2ml of chloroform, then add 1ml of the extract and sulfuric acid. A positive test and revealed by the appearance of a brown colour.

f.Carotenoid test: We Take 1ml of the extract, then add 1.5ml Hcl and a few drops of sulfuric acid. A positive test is revealed by the appearance of a green-blue color(Bouabid B, *et al.*,2016).

Determination of Phenolic Compounds:

Determination of total phenols: The determination of total polyphenols by the Folin-Ciocalteu reagent as described in 1965 by Singleton and Rossi. The coloration produced, whose maximum absorption is between 725 and 750 nm, is proportional to the number of polyphenols present in the plant extracts (Boizot N., *et al.,* 2006).

Determination of flavonoids: The quantification of the flavonoids was carried out by a method adapted by Zhishen *et al.,* (1999) with aluminium trichloride and sodium hydroxide. Aluminium trichloride forms a yellow complex with flavonoids and soda forms a pink complex absorbed in the visible range at 510 nm.

Determination of tannins: The condensed tannins are determined by the vanillin method in an acidic medium (Price M.L., *et al.,*1978). This method is based on the ability of vanillin to react with the condensed tannin units in the presence of acid to produce a colour complex measured at 500 nm. The reactivity of vanillin with tannins involves only the first unit of the polymer. The amounts of tannins are estimated using the vanillin method described by Julkunen-Titto (1985).

Determination of Antioxidant Activity:

The determination of the antioxidant activity by the DPPH radical was carried out according to the method of Dahmoune *et al.* (2003): 50 µl of extract added to 1.950 ml of the DPPH solution. The absorbance was read at 515 nm after incubation in the dark at 37 ° C for 30min. The percentage of inhibition of the DPPH radical is expressed by the formula:

DPPH (% inhibition) = ((Aa-Ab) / Ab) * 100; Aa: Control absorbance at 515 nm; Ab: Absorbance of the sample at 515 nm. IC50 or 50% inhibitory concentration is the concentration of the test sample necessary to reduce 50% of DPPH radical. The IC50s are calculated graphically using the plots of the percentages of inhibition as a function of different concentrations of the fractions tested.

Toxicity Test:

The toxicity test was carried out according to the 'predetermined dose' method of guideline 420 of (OECD, 2001) which consists of testing the alcoholic extract of the aerial part of *Inula viscosa* with a dose of 2000 mg /kg. The test was carried out on five females Westar albino rats, after an overnight fast (removal of food but not water). The extract is administered in a single dose using a gastric tube for appropriate intubation. Their behaviour was observed for 14 days after force-feeding (Sawadogo *et al.*, 2018).

RESULTS AND DISCUSSION

Ethnobotanical Study:

In general, the use of *Inula viscosa* in the Sidi Bel Abbes region is widespread among all age groups, with predominance in people aged over 60 (35%). According to the respondents, the use of *Inula viscosa* is also widespread among the 40 to 60-year-old age group with a rate of (34%) and (29%) in people aged 20 to 40 years, (Fig.2). In fact, the Knowledge of medicinal plants properties is generally acquired through long experience accumulated and passed down from one generation to the next. The transmission of this knowledge is in danger today because it is not always assured (Anyinam C. 1995).

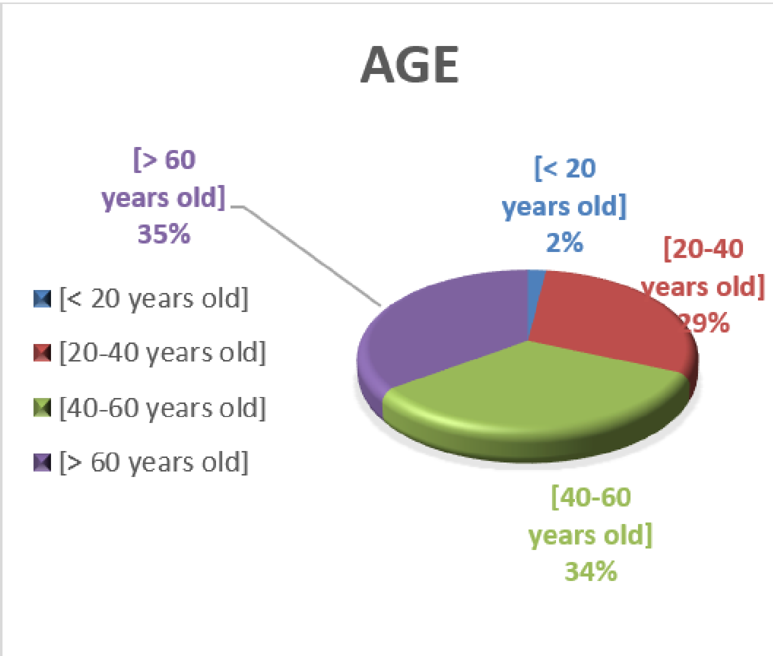

Fig. 2: Age groups of the population fromtheethnobotanical survey *of Inulaviscosa* uses

Results obtained according to the interview show that people, who belong to the age group of 40 to 60 years have more knowledge about plants than others. The use of herbal remedies varies by gender. Women use medicinal plants much more than men. In fact, 51% of the subjects questioned who use *Inula viscosa* are women and 49% of the population are men (Fig. 3). This can be explained by the use of medicinal plants by women in other areas than conventional therapy and by their responsibility as mothers (Bouzid A., *et al.*, 2017); when they prepare the recipes for family care members (Rhattas M., et al., 2016).

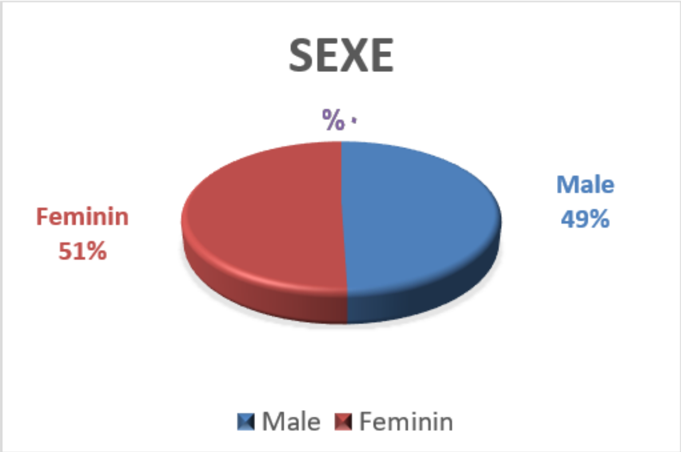

Fig. 3: Gender of the surveyed population from the ethnobotanical survey *of Inulaviscosa* uses

The vast majority of plant users have a university education (38%) and a secondary level (30%). These percentages are the intellectual confidence in herbal medicine and plants to heal themselves. However, people with primary education and illiterates have a significant percentage of plant use (16%) for each slice (Fig. 4). The majority of people who use herbal medicine do not take the advice of someone with expertise in this area.

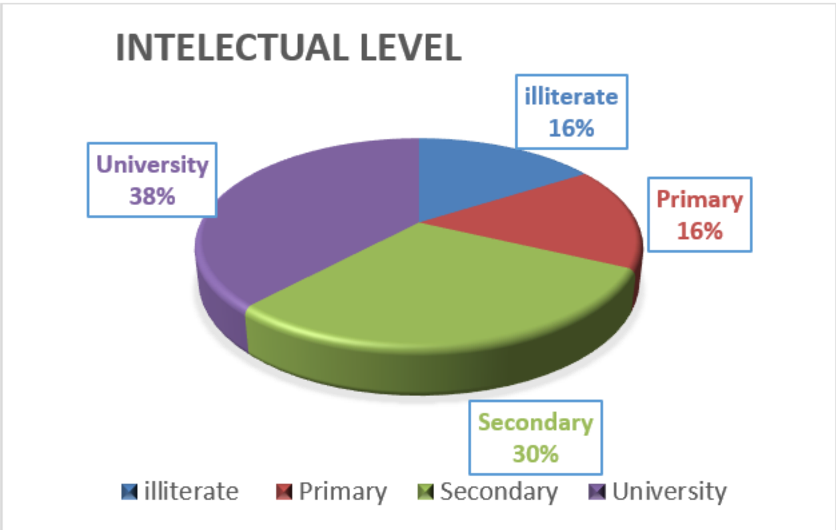

Fig. 4: Intellectual level of the surveyed population from the ethnobotanical survey *of Inulaviscosa* uses

We have collected information related to the various methods of traditional preparations which facilitate the administration of the active principle. Several methods of preparation are used, namely infusion, decoction. Most of these preparations would be taken orally and sometimes topically. Figure 5 shows that the infusion, the decoction, and the poultice are the most used methods of preparation with respective rates of 35%, 22%, and 20%. All of the remaining preparation methods, namely powder, fumigation, maceration, are presented by a cumulative rate (23%). Herbalists advise their clients to use this herb on wounds and other skin infections. The bath with maceration, infusion, or decoctions is also another route of administration when the time of treatment and the doses are very diverse. The use of certain preparations obtained from *Inula viscosa* would be undesirable for pregnant women causing abortive danger. In fact, it is reported that plants exhibiting toxic, teratogenic, and abortive effects generally have a bitter taste (Rodrigues E., 2007).

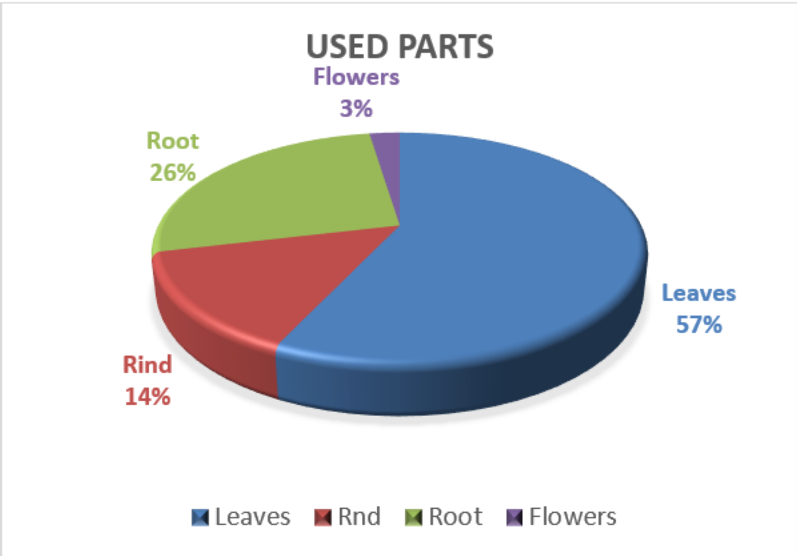

Fig. 5: Used parts of *Inulaviscosa* from the ethnobotanical survey *of Inulaviscosa* uses

This survey enabled us to identify several chronic diseases treated with *Inula viscosa*. The results obtained (Fig. 6) show that this plant is involved in the treatment of the following conditions: osteoarticular or rheumatic diseases (33%), dermatological conditions (33%), nervous system (11%), digestive disorders (11%), cardiovascular diseases (6%), the rest includes other diseases with a rate of (9%). Phytochemical tests are qualitative analyses that allow to detection of different classes of secondary metabolites that the plant contains. These reactions are based on phenomena of precipitation or coloration by specific reagents of each compound family.

The experimental results of our extracts summarised in Table 1 show the presence or absence of ~~certain~~ chemical groups. These tests are related to the intensity of the precipitation, turbidity, or coloration, which is proportional to the amount of the substance sought. From the results obtained in the table above, we noted that the *Inula viscosa* is rich in polyphenols, especially for the methanolic extract. Similarly, we recorded a significant presence of tannins with the three solvents (distilled water, ethanol, and methanol). Glucosides as ~~a~~ terpenoid are strongly positive in methanolic and aqueous extract and weakly positive in hexane extract and ethanol.

Flavonoids are moderately positive in hexane extract and methanol. They have a low presence in aqueous extract and ethanol for leaves and twigs. We can also conclude that hexane is the best solvent for the extraction of carotenoids, where we recorded a strong presence (+++) for both parts of the plant. Phenols are detected moderately in methanol for both organs and negative in other solvents.

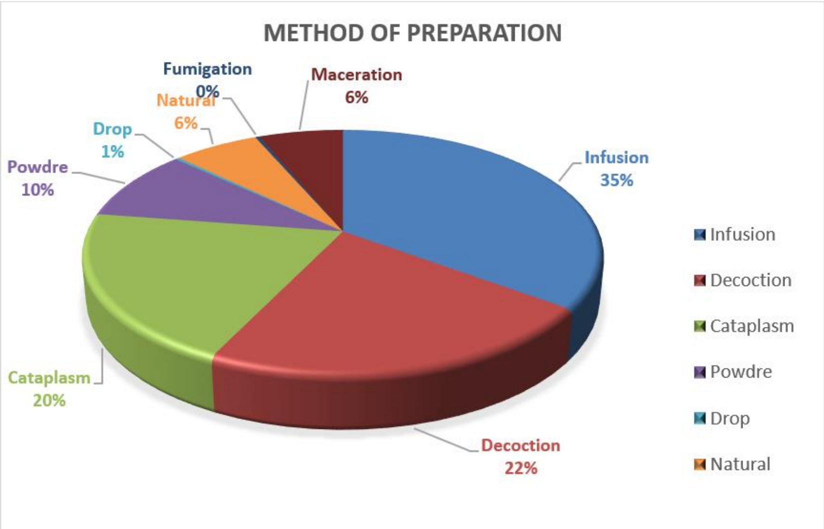

Fig. 6: Method of preparation from the ethnobotanical survey *of Inula viscosa* uses

Table 1: Characterization results of the different chemical groups in the leaves and stems of *Inula viscosa*.

Metabolits \ Organs	Leaves	stems
Hexane extract		
Terpenoidss	+	-
Tanins	-	-
Phenols	-	-
Flavonoids	+ +	+ + +
Glucosids	+	+ + +
Caroténoides	+ + +	+ + +
Ethanolic extract		
Terpenoidss	+	+
Tanins	+ + +	+ + +
Phenols	+	+
Flavonoids	+	+
Glucosids	+ + +	+ + +
Carotenoids	+	+
Methanolic extract		
Terpenoids	+ + +	+ + +
Tanins	+ + +	+ + +
Phenols	+ +	+ +
Flavonoids	+ +	+ +
Glucosids	+ +	+ + +
Carotenoids	+	+
Aqueous extract		
Terpenoids	+ + +	+++
Tanins	+ + +	+ + +
Phenols	-	-
Flavonoids	+	+
Glucosids	-	-
Carotenoids	-	+

Total Phenols:

The estimate of the total polyphenol content (Fig.7) of the various extracts was obtained according to the method using the Folin-Ciocalteu reagent. The results obtained were expressed in mg equivalent of gallic acid per gram of extract (mg GAE / g), using the linear regression equation of the calibration curve plotted for gallic acid according to ($y = 0{,}011x + 0{,}091$, $R^2 = 0{,}997$)

The results of the determination of total phenols in the aqueous extract show that the two organs contain a greater proportion (36.78 ± 4.78 mg AGE / g in leaves and 42.85 ± 1.05 mg AGE / g in stems), followed by the proportion in the ethanolic extract contained in leaves and stems (31.45 ± 2.35 mg AGE / g for leaves and 30.28 ± 0.13 mg AGE / g for stems).

On the other hand, the methanolic extract represents the lowest polyphenol content in the two organs (7.91 ± 0.37 mg AGE / g for leaves and 4.83 ± 1.14 mg AGE / g for Stems), as well as for hexane which shows a low content of total phenols in the twigs (6.81 ± 0.08 mg AGE / g). This content is higher in leaves but remains low compared to other extracts (17.66 ± 3.00 mg AGE / g).

Given the results obtained, the highest concentrations of total phenols are recorded in the aqueous and ethanolic extract of the two organs. The concentration of polyphenols in the ethyl acetate extract was more interesting in the leaves (95.47 ± 4.7 mg AGE / g) compared to the twigs (73.49 ± 2.2 mg AGE / g) and the cones (61.62 ± 5.7 mg AGE / g). By comparing the results concerning the leaves with those of the work carried out by Benaïssa Keddar (2014) where the concentration of total phenols in the leaves was (85.20 ± 0.07 mg AGE / g). We found that the total phenols here are lower. This difference may be due to the effect of climatic stress.

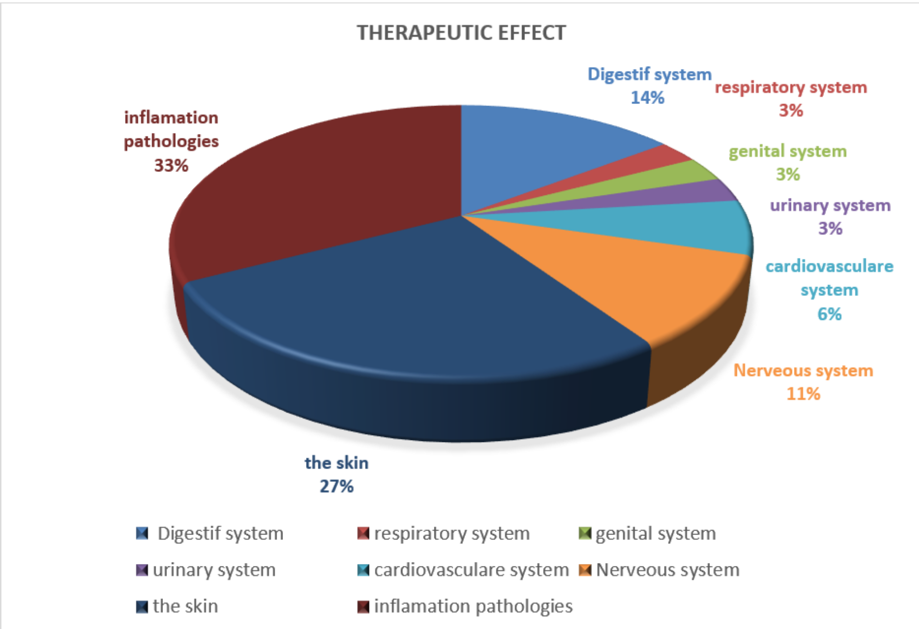

Fig. 7: Treated diseases from the ethnobotanical survey *of Inulaviscosa* **uses**

Flavonoids:

The quantitative determination of the flavonoids in the four extracts was made according to a linear calibration curve ($y = 0{,}007x + 0{,}148$, $R^2 = 0{,}984$) carried out by a standard solution of catechin at different concentrations, from which the flavonoids content of the two organs of the plant and which is expressed in mg equivalent of catechin per gram of extract (mg EC / g). The results obtained were presented in the histogram of Figure 8.

We note that the ethanol extract of the leaves contains the highest content of flavonoids (133.93 ± 3.12 mg CE / g) followed by that enclosed by the aqueous extract of the leaves (61, 17 ± 3 96 mg CE / g) and that the same extract of stems (51, 61 ± 12, 87 mg CE / g). The concentration of flavonoids contained in the aqueous extract ~~is~~ observed in the stems is low (37.12 ± 1.09 mg CE / g) concerning the leaves.

Furthermore, the results of the methanol flavonoids in leaves (24.35 ± 1.70 mg CE / g) and in stems (5.04 ± 2.13 mg CE / g) are low. Same for the extract of hexane which is low especially in stems (10.64 ± 0.33 mg CE / g). We can deduce that the average concentration of flavonoids contained in the leaves is higher than that in stems which are justified by the works of (Benaïssa Keddar, 2014) where we found a high concentration of flavonoids in leaves.

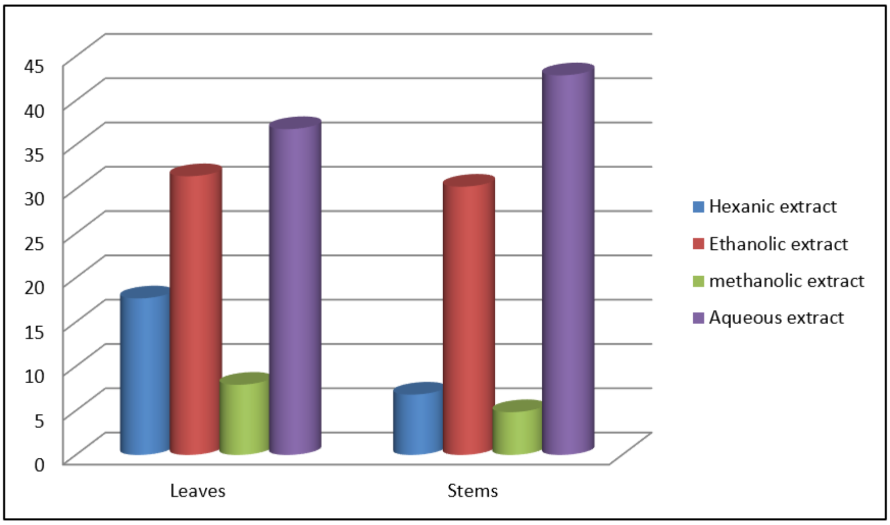

Fig. 8: Concentrations of polyphenols in leaves and twigs of *Inula viscosa* (mg GAE /g).

Condensed Tannins:

The content of the condensed tannins was obtained from a calibration curve established with increasing concentrations of catechin ($y = 0.000199x + 0.000618$, $R^2 = 0.995$). The results were expressed in mg equivalent of catechin per gram of extract (mg CE / g). The histogram below shows the results obtained (Fig. 9).

The content of condensed tannins in the hexane extract of leaves is very high (638.52 ± 10.64 mg EC / g), followed by that of the ethanolic extract of the same organ (363.41 ± 37.12 mg EC / g), then the methanolic extract in the leaves (75.80 ± 5.04 mg EC / g) and with almost the same content of the ethanol extract (79.57 ± 0.68 mg EC / g) and methanol (68.13 ± 16.24 mg EC / g) from stems which are also very weak.

The content of condensed tannins in the other extracts is low and almost non-existent compared with the hexane extract; the content of condensed tannins is (5.81 ± 0.86 mg EC / g) for the aqueous extract of the leaves. For hexane extracts and water are respectively: (12.77 ± 0.45 mg EC / g) and (1.66 ± 0.16 mg EC / g). We can notice that the condensed tannins are too high in the leaves than in the stems of *Inula viscosa*.

The amount of total phenols, flavonoids, and condensed tannins is variable between the leaves and stems of our studied species. The leaves and stems are rich in total phenols, while the leaves are particularly rich in flavonoids and condensed tannins compared to the stems, which have more or less low contents.

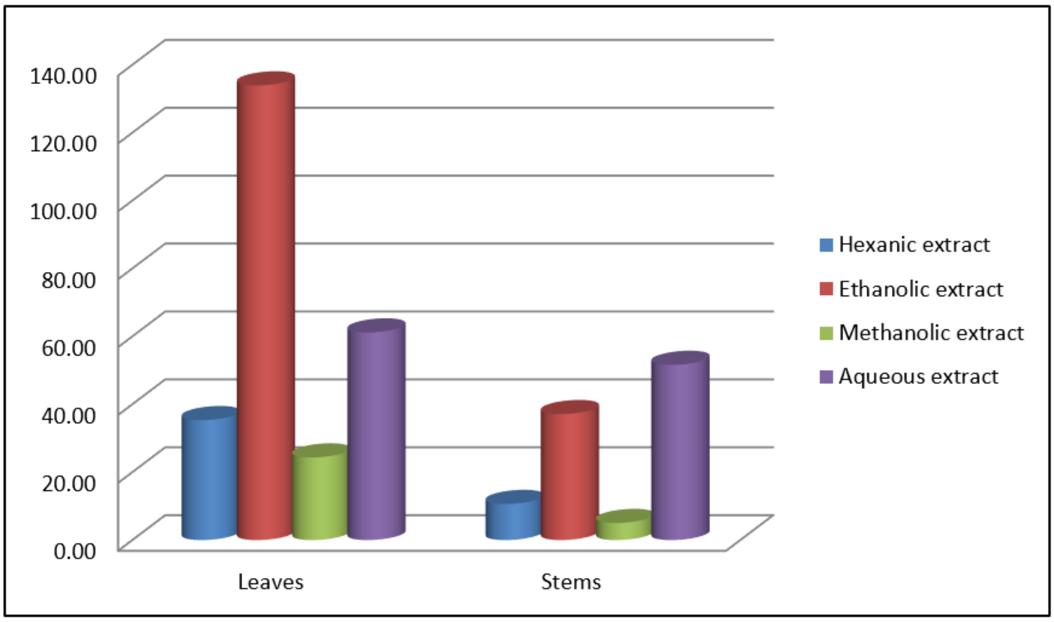

Fig. 9: Concentrations of flavonoids in leaves and stems of *Inula viscosa* (mg EC / g).

Antioxidant Activity:

The results of the anti-radical activity with DPPH are represented by the percentages of inhibition for each concentration as well as the values of the 50% inhibition concentration (Fig. 10). For each extract, we determined the IC50 value which is the concentration of the tested sample necessary to reduce 50% of the DPPH radical. The IC50 are calculated graphically by the linear regressions of the plotted graphs.

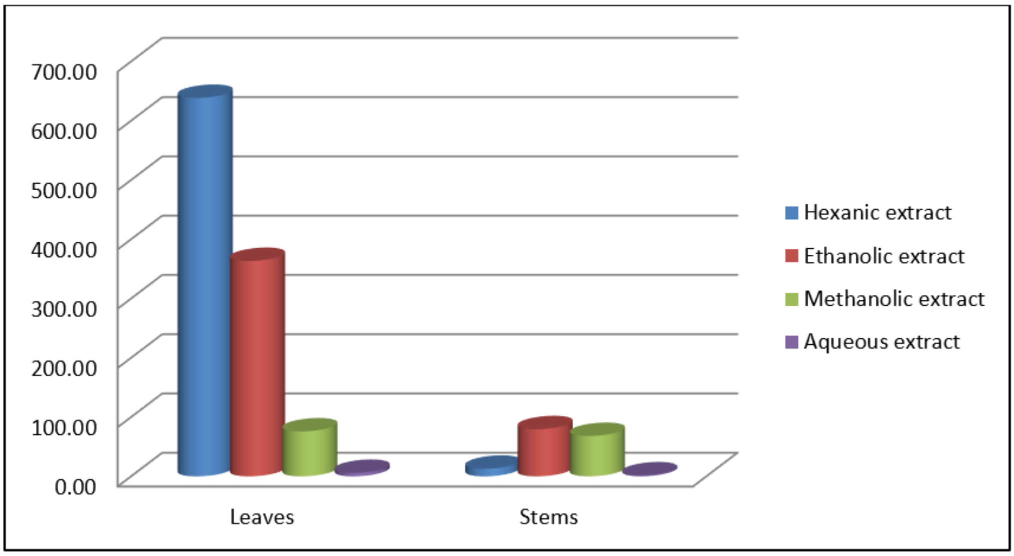

Fig. 10: Concentrations of condensed tannins in leaves and stems of Inula *viscosa* (mg EC / g).

Toxicity Test:

Oral administration of a single 2000 mg/kg dose of the alcoholic extract of the aerial part of *Inula viscosa* did not cause death or signs of toxicity in rats. The alcoholic extract of the aerial part of *Inula viscosa* is non-toxic for a dose of 2000 mg/kg or less. And the 50% lethal dose (LD50) orally is greater than 2000mg/kg.

Conclusion

The ethnobotanical investigation carried out on the *Inula viscosa* uses field confirms that medicinal plants are still a source of medical care. Several identified uses can be considered as new, including the use against digestive disorders and cardiovascular disease. The survey revealed a multitude of results on *Inula viscosa* uses, which is closely linked to the profile of the people surveyed. Thus, young people, compared to the elderly, generally do not know the usefulness of the plant. Older people have provided more reliable information, as they hold much of the ancestral knowledge that is part of oral tradition.

The results obtained show that this plant is involved in the treatment of osteoarticular or rheumatic disorders (33%) and dermatological disorders (33%). Women and men have shared medicinal knowledge, with a slight advantage going to women. From the phytochemical screening on the different preparations of aqueous, methanolic, ethanolic extracts, and hexane extracts from the aerial parts studied (leaves and stems), we concluded that *Inula viscosa* is rich in flavonoids, terpenoids, tannins, glycosides, and carotenoids.

Throughout this work, we have shown that the yield of phenolic compounds is more important in leaves compared to stems. The amount of total phenols, flavonoids, and condensed tannins is variable between the different parts of *Inula viscosa*. Thus, the aqueous extract of the leaves and stems rich in total phenols (36.78 mg AGE / g and 42.85 mg AGE / g) compared to the organic extracts, with very high concentrations and which are respectively (36.78 mg AGE / g of extract and 42.85 mg AGE / g of extract). The amount of flavonoids contained in the leaves is higher with (133.93 mg EC / g) than stems, while the leaves appear too rich in condensed tannins (638.52 mg EC / g) of extract compared to stems which have more or less low contents.

The extracts prepared have interesting and variable reducing power depending on the plant part used and the solvent chosen for the extraction. They recorded remarkable IC50 values of 0.1 mg / l to 4.1 mg / l.

Further in-depth studies are needed and can be summed up in the following points:

- Isolation and characterization of the active compounds in the different extracts by more specific methods
- Evaluation of other biological effects in vitro as in vivo of crude extracts.

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