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Antioxydant activity of *Silybum marianum and Ajuga iva* natural dyes.

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***Abstract*—**

As part of an optimization of the extraction of natural antioxidants, we proposed to investigate the richness of natural dyes (polyphenols, flavonoids, and condensed tannins) of the 3 fractions of different polarities (methanol, acetone and chloroform) and explore the variability of biological activities including antioxidant activity through various tests. The results showed significant variability of the levels of phenolic compounds and their antioxidant capacity. This variability has shown a remarkable superiority of the methanol fraction having the highest antioxidant levels including total polyphenols (35.60 ± 0.85 mg EAG / mg MS) and flavonoids (8.31 ± 1 03 µg EQ / mg MS) for *Silybum marianum* (thisle). In addition, this same fraction had the best antiradical activities with the lowest IC_{50} values (6 mg/mL). Thus, extracts of thistle could be used as remarkable antioxidants acting at low dose. Such a study could be useful to enhance the thistle as a source of bioactive molecules.

Keywords: *Silybum marianum*, natural dyes, solvent effect, total antioxidant activity.

I. Introduction

Medicinal plants remain an inexhaustible source of biologically active substances. In most Southern countries, these plants constitute a fundamental component of the health system where their use can take many forms (dried plants to prepare herbal teas, essential oils, officinal tinctures, fluid extracts and dry extracts). In Tunisia, the climatic diversity, soil and plant breeding have promoted the development of flora (estimated at about 2100 species) housing in over 149 medicinal species, 38 herbs where 80 species are subjected to intensive crops.

Currently, vegetable remedies prevailed despite the development of modern medicine. Therefore, the research of natural antioxidants in recent years was a huge progress (Prasad et al., 2009), especially polyphenols that are the most active class (Oueslati et al., 2012). Natural antioxidants are highly recommended to replace the synthetic antioxidants currently used in therapy and diet (Gulcin et al. 2004), who are accused of being long-term toxic and even carcinogenic (Ajila et al., 2007). Thus, in the context of the valorization of natural bioresources, thistle (*Silybum marianum*) and ivette (*Ajuga Iva*) were chosen.

The purpose of this study was **(i)** to determine the potential effect of pure solvents on the extraction of natural dyes of the two selected plants and their antioxidant capacity. **(ii)**. After choosing the solvent for efficient extraction, the anti-radical activity of the extracts of the various organs of the thistle was determined.

II. Materials and Methods

1) Plant material

Our study was performed on the different organs of the Thistle and Ivette (Fig.1) which were harvested from plants in March-April 2014 grown in Tunis (Sidi Thabet (Latitude is 36.913900000000010000, The Longitude is 10, 036100000000033000). After that, the samples were freeze-dried, then ground to fine powder by an electric mill and conserved in a desiccator at room temperature (~25° C) in darkness for further uses.

(a) **(b)**

Fig.1. Whole plants of thistle (a) and ivette (b).

2) Determination of Phenolic Compounds

2.1. Determination of Total Polyphenol Contents

Determination of Total Polyphenol Contents.Colorimetric quantification of total phenolics was determined, as described by Dewanto et al. 14 Briefly, 125µL of suitable diluted sample extract were dissolved in 500µL of distilled water and 125µL of Folin Ciocalteu reagent. The mixture was shaken, before adding 1250µL of Na2CO3 (7 g/100 mL), adjusting with distilled water to a final volume of 3 mL, and mixed thoroughly. After incubation for 90 min at 23 C in darkness, the absorbance versus a prepared blank was read at 760 nm. Total phenolic contents of flower were expressed as mg gallic acid equivalents per gram (mg GAE/g) through the calibration curve with gallic acid.

2.2. Determination of Flavonoid Contents.

Total flavonoid contents were measured according to Dewanto et al. (2002). An aliquot of diluted sample was added to 75µL of $NaNO_2$ solution (5%), and mixed for 6 min, before adding 0.15 mL of $AlCl_3$ (10%). After 5 min, 0.5 mL of 1 M NaOH solution was added. The final volume was adjusted to 2.5 mL, thoroughly mixed, and the absorbance of the mixture was determined at 510 nm. Total flavonoid contents of flower extracts were expressed as mg catechin equivalents (CE) per gram of dry weight respectively (mg GAE/g DW).

2.3. Quantitative Determination of Condensed Tannins

In the presence of concentrated HCl, condensed tannins were transformed by the reaction with vanillin to anthocyanidols. 50µL of the extract appropriately diluted was mixed with 3 mL of vanillin (4%) and 1.5 mL of HCl. After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents of flowers were expressed as mg catechin equivalents (CE) per gram of dry weight through the calibration curve with catechin.

3. Evaluation of antioxidant activity

3.1. Total antioxidant capacity

A dose of 200 µl of plant extract was added to 2 ml of the solution at acidic pH containing sulfuric acid (H_2SO_4; 0.6M) sodium phosphate (NaH_2PO_4, H_2O; 28 mM) and the ammonium heptamolybdate ($(NH_4)\ 6MO_7O_{24}$, $4H_2O$; 4 mM). The mixture is then incubated at 95 ° C for 90 min. After cooling to room temperature, the absorbance is measured at 695 nm. The total antioxidant activity is expressed as mg gallic acid equivalent per gram of dry matter (mg g^{-1}MS EAG).

3.2. DPPH assay

The electron donation ability of the methanol extracts was measured by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato29. One half mL of 0.2 mM DPPH methanolic solution was added to extracts (2 mL, 10-1000 µg mL−1). After an incubation period of 30 min at room temperature, the absorbance was against a blank at 517 nm. The inhibition percentage of free radical DPPH (IP%) was calculated as follow:

$$IP\% = ((A_{blank} - A_{sample})/A_{blank}] \times 100.$$

Where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the regression equation prepared from the concentration of the extracts and the inhibition percentage. BHT, a synthetic antioxidant used generally for food, cosmetics and pharmaceuticals was used as standard.

III. Results and Discussions

III.1. Extracts yields

The mass yield was calculated after elimination of solvents: methanol, chloroform and acetone. After evaporating the solvent, the residue obtained was pale green color (Table 1).

Table 1. Mass yield of extracts of thistle and ivette (%).

	MET	ACT	CHL
Thistle: Leaves	11	1	3
Thistle: Flower	7	2	3
Thistle: Stem	7	1	1
Ivette: Leaves	10	2	3
Ivette: Stems	7	2	1

MET: Methanolic extracts
ACT: Acetonic extracts
CHL: Chloroformic extracts

It is noted from the results in Table 1 that only the methanol extracts gave the best yield varying between 7% and 11%, while for other acetone and chloroform extracts, poor yields don't exceed 3%.

III.2. quantitative analysis of phenolic compounds

We proceeded to the determination of polyphenols and total flavonoids and condensed tannins extracts from different organs of the thistle and ivette. The results were shown in Table 2.

The total polyphenol content of the methanol extract (ranging from 22.19 to 35.60 mg g^{-1} EAG MS for thistle and 7.11 to 21.47 mg g^{-1} EAG MS for ivette) was more important than the other extracts. Hence, extracts of the stems and leaves of *Silybum marianum* were a promising source of phenolic compounds. Similarly, the ivette leaves were characterized by their high polyphenol content compared to stems. Concerning flavonoids, they were detected in small amounts for the two selected plants. For thistle, the methanol extracts of the stems (8.31 mg EC g^{-1} DW) contain more flavonoid contents than the other parts of the plant. As for polyphenol and flavonoid contents, the methanol fraction was distinguished by its richness of condensed tannins compared to the two remaining fractions.

The polyphenol content of thistle was higher than other species of the same family cited in the literature, the leaves of *Achillea millefolium* (9.55 mg g^{-1} EAG DW), the aerial part of *Artemisia vulgaris* (3.83 mg EAG g^{-1} DW) and leaves of *Tanacetum vulgare* (1.68 mg g^{-1} EAG DW) (Wojdyło et al., 2007).

Thus, the extraction of phenolic compounds is influenced by their chemical nature, the used extraction method and mainly the nature of the extraction solvent. Indeed, Naczk and Shahidi (2004) showed that the solubility of the polyphenols was determined by the polarity of the solvent, the polymerization degree of these phenolic substances and their interactions.

III.3. Evaluation of antioxidant extracts of thistle

III.3.1. Total antioxidant activity

By comparing results to the trolox, it was clearly noted that the three methanolic extracts (leaf, flower and stem) have significant total antioxidant activity. At a concentration of 1000 mg / ml, there is a maximum absorbance equal to 0.67 for methanol in the leaves, while it is 1.2 in flowers and 0.68 for stems. The absorbance was significantly lower for the acetone and chloroform extracts at a concentration of 1000 µg / ml (Figure 2).

It was confirmed that the methanol extracts (leaf, flower and stem) have the most significant antioxidant activity as compared to those of acetone and chloroform.

Table 2. Total polyphenols, flavonoids and condensed tannins contents of thistle and ivette depending on the solvent.

	Phenolic contents [1]			Flavonoid contents [2]			Condensed tannin contents [3]		
	MET	ACT	CHL	MET	ACT	CHL	MET	ACT	CHL
Thistle: Leaves	32.17±1.39	1.09±0.24	2.02±0.85	3.90±0.54	0.42±0.11	0.725±0.11	15.72±1.47	3.20±0.05	9.39±0.50
Thistle: Flowers	22.19±0.36	6.39±0.29	9.70±0.15	3.82±0.87	0.97±0.20	1.25±0.34	1.97±0.44	1.63±0.55	2.77±0.07
Thistle: Stems	35.60±0.85	1.80±0.20	3.57±0.66	8.31±1.03	0.65±0.10	1.83±0.20	2.46±0.76	1.05±0.22	0.16±0.034
Ivette: Leaves	21.47 ±2.87	4.95 ± 1.27	7.61 ± 0.52	3.26 ± 0.86	0.23 ± 0.08	0.20 ± 0.09	13.93 ±0.76	2.69 ± 0.19	3.79 ± 0.18
Ivette: Stems	7.11 ± 0.68	2.57 ± 0.41	0.80 ±0.010	0.89 ±0.26	0.17 ± 0.07	0.066 ± 0.013	3.98 ± 0.60	1.79 ± 0.15	0.49 ± 0.07

[1] µg EAG/mg DW. [2] µg EQ/mg DW. [3] µg ECAT/mg DW

III.3.2. III.3.2. radical scavenging activity

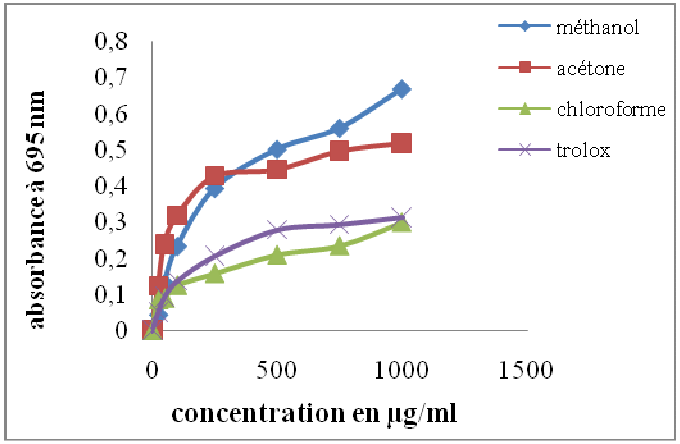
The scavenging power of DPPH was determined by following the decrease in absorbance at 517 nm which is induced by the antioxidants that act as hydrogen donors (Soares et al ., 1997).

From Figure 3. The percent inhibition (PI) of DPPH radicals was proportional to the concentration of the medium in methanolic extracts. So. they are capable of releasing groups having a high capacity to reduce these free radicals to an IC_{50} of 6 mg / mL. These three extracts could be used as remarkable antioxidants acting at low dose.

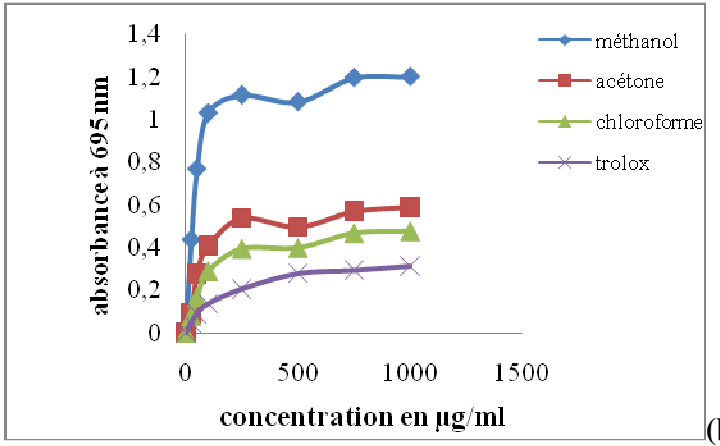
In a general way. phenolic compounds are known for their role in the prevention of certain diseases due to their richness in antioxidants and their anti-radical effect. Because of the diversity of their chemical structures. These compounds are likely to have very different antioxidant capacities (Dapkevicius et al ., 2002).

IV. Conclusion

From results obtained in this study, Yvette and thistle can be considered as promising sources of natural bioactive molecules that can be substituted to synthetic ones.



(a)



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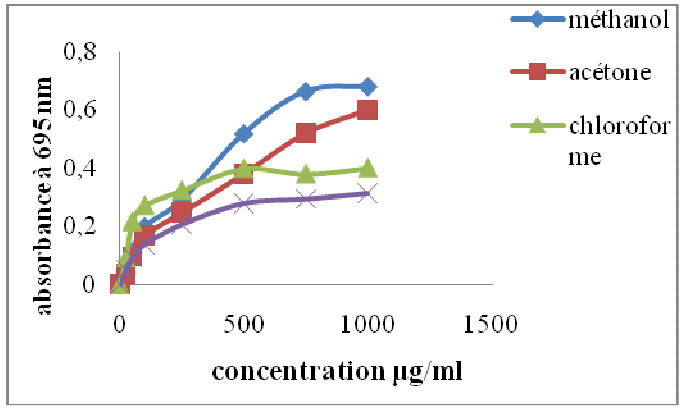

(c)

Figure 2. Total antioxidant activity of the leaves (a), flowers (b) and stems (c) of thistle depending as a function of solvents.

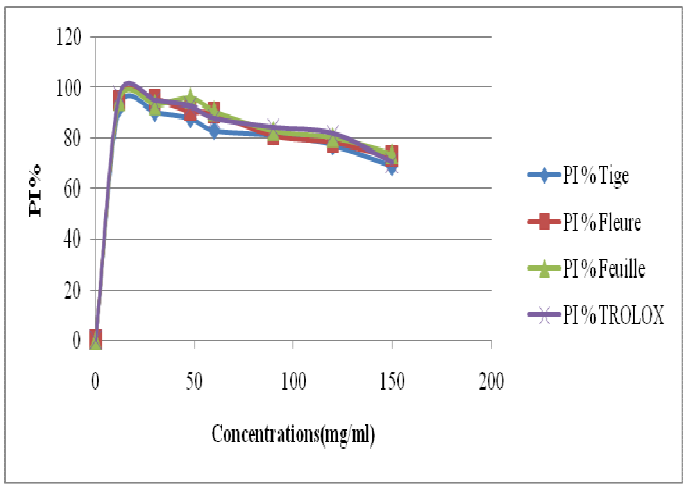

Figure 3. PI variation depending on the concentrations of methanol extract of different organs of the thistle.

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