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Evaluation of antioxidant and antiproliferative activities of dyeing plants

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Abstract.- Natural dyes are nowadays re-investigated but few data are available about their biological properties. In this study, ten dyeing plants from several genera (belonging to Anacardiaceae, Asteraceae, Betulaceae, Resedaceae, Rosaceae, Rubiaceae) were investigated for both antioxidant and antiproliferative activities. Aqueous crude extracts containing flavonoids associated with either phenolic acids or tannins were particularly antioxidant whereas no extract seemed to present antiproliferative properties suggesting a potential absence of toxicity. Primary pharmacological data from this study are discussed in relation with dyeing properties of plant species in order to highlight the uses of multi-functionalised natural products.

Key words : dyeing plant - antioxidant activity - antiproliferative activity - multifunctionalised extracts.

Résumé.- Les colorants végétaux connaissent actuellement un regain d'intérêt mais peu de données sont cependant disponibles concernant leurs activités biologiques. Au cours de ces travaux, les activités antioxydante et antiproliférative d'extraits aqueux de dix plantes tinctoriales appartenant à différents genres (des familles Anacardiaceae, Asteraceae, Betulaceae, Resedaceae, Rosaceae, Rubiaceae) ont été évaluées. Les extraits contenant des flavonoïdes associés à des acides phénoliques ou des tannins se sont révélés très antioxydants. De plus, aucun extrait n'a montré d'activité sur la prolifération cellulaire, suggérant ainsi une absence probable de toxicité. Ces données pharmacologiques préliminaires, associées aux propriétés colorantes de ces espèces, sont discutées afin de souligner l'opportunité d'utiliser ces extraits naturels à propriétés multiples.

Mots clés : plantes tinctoriales - activité antioxydante - activité antiproliférative - extraits multifonctionnels.

I. INTRODUCTION

Natural raw phytomaterials are nowadays of great interest because of increased consumer awareness and popular demand for natural and safer products. More particularly, natural dyes uses are particularly relevant today due to their attractiveness in terms of minimal environmental and health impact versus synthetic dyes, which have been suggested as sources of human diseases as skin cancer or allergic contact dermatitis (Anliker *et al.*, 1988; Moreau & Goossens, 2005). In consequence, studies have already focused on dyeing plants that represent alternative products to synthetic dyes. Subsequently, literature provided many data dealing with molecular isolation of dyeing molecules (Derksen *et al.*, 1998; Cristea *et al.*, 2003; Oberthür *et al.*, 2004; Guinot *et al.*, 2008; Peggie *et al.*, 2008), agronomic evaluation (Angelini *et al.*, 1997, 2003; Hartl & Vogl, 2003), extraction of pigments (Cadoni *et al.*, 2000; Cerrato *et al.*, 2002; Badami *et al.*, 2007) or dyeing process (Beltrame *et al.*, 1998; Kamel *et al.*, 2005; Vankar *et al.*, 2007). Nevertheless, little information is available regarding biological activity of natural dyeing extracts. In this perspective one plant was particularly investigated, Rubia tinctorum, because of the mutagenic potential of some of its phytochemicals (Kawasaki *et al.*, 1992; Jager *et al.*, 2006). To our knowledge, the work carried out by Singh *et al.* (2005) on antimicrobial activities is the only explorative and comprehensive study reported on biological properties of several natural dyes.

Consequently, in order to describe potential biological activity of such natural raw materials, we decided to study ten dyeing plants known for their dyeing properties as well as their medicinal value (Table I). Choice was based on our previously reported work (Guinot *et al.*, 2006). Antioxidant activity characterization was first assessed for both therapeutic and dyeing interests. In order to further consider the benefit of natural dyes versus synthetic dyes, antiproliferative activity was then evaluated on the same traditional dyeing extracts.

Table I.- Medicinal uses and phenolic content of the ten selected dyeing plants.
Tableau I.- Utilisation médicinale et contenu polyphénolique des dix plantes tinctoriales étudiées.

Dyeing plants	Medicinal uses	Polyphenolic content (Guinot *et al.*, 2006)
Alnus glutinosa	astringent, antipyretic, healing agent, antirheumatism (Valnet, 2001), diuretic, vermifuge (Bonnier, 1934)	flavonoids, tannins, caffeic derivatives
Artemisia vulgaris	antidiabetic (Villasenor & Lamadrid, 2006), emmenagogue (Bruneton, 1999), antispasmodic, vermifuge (Carnat *et al.*, 2000)	caffeic derivatives, flavonoids,
Coreopsis tinctoria	emètic, treatment of diarrhoea (Foster & Duke, 1990)	flavonoids
Filipendula ulmaria	antibacterial (Rauha *et al.*, 2000), anti-inflammatory, astringent, healing agent (Krasnov *et al.*, 2006)	tannins, flavonoids, phenolic acids
Reseda luteola	antiworm (Bonnier, 1934), anti-inflammatory (Johansson *et al.*, 2002)	flavonoids
Rhus typhina	antidiabetic (McCune & Johns, 2002)	flavonoids, tannins
Rubia tinctorum	antifungal (Manojlovic *et al.*, 2005), antimicrobial (Kalyoncu *et al.*, 2006)	anthraquinones
Serratula tinctoria	antiviral, anti-inflammatory (Huang *et al.*, 2004)	flavonoids
Solidago canadensis	antiphlogistic, treatment of nephritis, cystitis, urolithiasis, rheumatism (Apati *et al.*, 2003)	flavonoids, caffeic derivatives
Tagetes patula	antifungal (Mares *et al.*, 2004)	flavonoids

II. MATERIALS AND METHODS

A. Plant materials

Whole aerial parts of *Coreopsis tinctoria* Nutt. (Asteraceae), *Reseda luteola* L. (Resedaceae) and *Artemisia vulgaris* L. (Asteraceae), leaves of *Alnus glutinosa* (L.) Gaertn. (Betulaceae), *Rhus typhina* L. (Anacardiaceae) and *Serratula tinctoria* L. (Asteraceae), head flowers of *Filipendula ulmaria* (L.) Maxim. (Rosaceae) and *Solidago canadensis* L. (Asteraceae), flowers of *Tagetes patula* L. (Asteraceae) and roots of *Rubia tinctorum* L. (Rubiaceae) were provided from Le Jardin conservatoire des plantes tinctoriales (association Couleur Garance, Lauris, France). Plants were directly air-dried after harvesting in a well ventilated room and then transported to the laboratory.

B. Tested materials

Air-dried plant materials were ground using a domestic blender. Extraction was carried out once by decoction in distilled water (5%; w/v): the suspensions were heating up to 100 °C under magnetic stirring and boiling was kept for 10 minutes. After passing through filter paper, crude extracts were adjusted to initial volume with distilled water. Plant extractions were made in triplicate for biological test replication.

C. Antioxidant and antiproliferative activities evaluation

Antioxidant activity was evaluated on crude extracts using an antioxidant assay kit simulating enzymatic oxidation (Cayman Chemical Company, USA; Halliwell, 1996). Results were expressed in mM Trolox equivalent by interpolation on an external calibration curve using Trolox standard solutions in a range from 0 to 0.330 mM.

Antiproliferative activity was carried out on L1210 mouse leukemia cells and IC_{50} (50% inhibitory concentration expressed in mg of dried plant/ml of cell culture medium) was determined comparing results with those obtained in an assay containing no plant extract as previously described (Rivière *et al.*, 2006). Two dyeing standard were used in order to compare antiproliferative activities of natural dyes versus synthetic dyes: a plant agroalimentary dye used in industry (E163 from *Daucus carota* L. var. *nigra*, black carrot) and a synthetic textile dye now forbidden because of its mutagenic toxicity (Congo red, CI 22120, direct red 28).

Both biological tests were performed in triplicate using new fresh plant extracts.

III. RESULTS

A. Antioxidant activity evaluation

Three groups of plant extracts could be distinguished based on antioxidant activity results, as shown in Table II. All ten plant extracts demonstrated antioxidant capacity superior to 1 mM Trolox equivalent, Trolox being the hydrosoluble form of vitamin E commonly used as antioxidant reference.

Table II.- Antioxidant activity of plant extracts.

Tableau II.- Activité antioxidante des extraits de plantes.

Antioxidant activity (mM Trolox equivalent)	Plants
Below 2 mM	*Reseda luteola*
	Rubia tinctorum
	Serratula tinctoria
Between 2 mM and 4 mM	*Artemisia vulgaris*
	Coreopsis tinctoria
	Tagetes patula
Over 4 mM	*Alnus glutinosa*
	Filipendula ulmaria
	Rhus typhina
	Solidago canadensis

B. Antiproliferative activity evaluation

It is considered that antiproliferative activity, from raw material extract, on cultured cell line is

significant for IC_{50} < 0.025 mg/ml (Lohézic-Le Dévéhat *et al.*, 2002). Regarding our reference compounds, the authorised agroalimentary dye E163 did not demonstrate antiproliferative activity with an IC_{50} value of 3.27 mg/ml whereas the forbidden dyeing standard (Congo red) presented potential antiproliferative activity with an IC_{50} value of 0.04 mg/ml close to significance.

Table III.- Antiproliferative activity of plant extracts. [a] relative standard deviation < 10%.
Tableau III.- Activité antiproliféatrice des extraits de plantes.

Plants	Antiproliferative activity [a] (IC_{50} mg.ml^{-1})
Alnus glutinosa	0.21
Rhus typhina	0.25
Filipendula ulmaria	1.54
Artemisia vulgaris	1.89
Tagetes patula	1.91
Coreopsis tinctoria	1.95
Solidago canadensis	2.32
Serratula tinctoria	2.41
Reseda luteola	16.00
Rubia tinctorum	25.00

Regarding our plant extracts (results showed in Table III) none of them presented antiproliferative effect when compared to literature standard. Extracts with IC_{50} < 1 mg/ml (of dried plant in cell culture medium) were obtained from *Alnus glutinosa* and *Rhus typhina* (average IC_{50} = 0.23 mg/ml) remaining 10 times below antiproliferative threshold dose. All other extracts (except those from *Reseda luteola* and *Rubia tinctorum*) were characterized by IC_{50} from 1.54 to 2.41 mg/ml (average IC_{50} = 1.68 mg/ml), in a range closer to IC_{50} from authorized dye E163. Furthermore *Reseda luteola* and *Rubia tinctorum* showed IC_{50} of respectively 16 mg/ml and 25 mg/ml (average IC_{50} = 20.5 mg/ml) clearly indicating total absence of any antiproliferative action. In addition it should be noted that beside antiproliferative effect assessment, none of the ten aqueous plant extract demonstrated any deleterious influence on cultured cells.

IV. DISCUSSION

Phyto-products from natural source are nowadays widely requested by consumers because of environmental and safety issues. Therefore textile, agroalimentary, cosmetic and even pharmaceutical industries are turning back to natural products. These materials are supposed to fulfil three major expectations: (1) to be respectful of the environment since they are issued from it, (2) to demonstrate expected bioactivity (dyeing, food conservative, health promoter) and (3) to be more biocompatible therefore safer than synthetic compounds. This trend is reinforced by international legislation and projects such as the European REACH program (Registration Evaluation Authorization Restriction of CHemical substances) (website: *ec.europa.eu/environment*). Indeed REACH is intended to improve protection of human health and environment, therefore promoting alternative to synthetic toxic compounds.

The ten aqueous extracts we tested were obtained from plants traditionally used as natural dyes. These vegetals are typically the kind of agro-resources that might be re-valorised through various industrial pre-cited uses and in first instance as dyeing agents. They are nowadays re-investigated for industrial dyeing raw material purpose. In this context, bioproducts were originally left aside (for synthetic compounds) because of their poor light fastness until toxicity of synthetic molecules rose. As oxidative mechanisms are particularly quoted to be responsible for dyes photodegradation (Giles & McKay, 1963), the use of dyeing plant extracts with anti-oxidative potency becomes of great interest. All the plants we worked on showed antioxidant capacity over 1 mM Trolox (vitamin E) equivalent. In accordance with previous reports (Kumarasamy *et al.*, 2006; Hu, 2004; Calliste *et*

al., 2001; Apati *et al.*, 2003), *Alnus glutinosa, Filipendula ulmaria, Rhus typhina* and *Solidago canadensis* demonstrated even higher antioxidant properties. As mentioned in Table I, these plants contain flavonoids associated with other polyphenolic derivatives, *i.e.* tannins or phenolic acids, which are well known for their antioxidant potential (Kumagai *et al.*, 2003; Sroka & Cisowski, 2003). Elevated antioxidant status associated with dyeing properties is a combination of interest to improve plant raw material for use as stable colouring as previously reported (Cristea & Vilarem, 2006). In our case we propose to select plants with dyeing properties that produce themselves, without external addition, their own high internal antioxidant protection system.

Bioactivity, *i.e.* antioxidant, being demonstrated for all plants studied, the next point for dyeing, as well as for therapeutic or even agroalimentary purpose, lays on the basic yet fundamental need for safety. The main primary parameter to consider in this respect was putative cytotoxicity assessed through an antiproliferative assay on a cultured mouse cell line (Table III). Our selected plants did not demonstrate any antiproliferative activity (all $IC_{50} > 0.025$ mg/ml; Lohézic-Le Dévéhat *et al.*, 2002) on cultured cells. This result suggests that all plant extracts were not cytotoxic, with an average IC_{50} of 5.34 mg/ml and a minimal value of 0.21 mg/ml well above the antiproliferative action threshold defined by Lohézic-Le Dévéhat *et al.* In addition even the lowest IC_{50} stayed 5 fold more elevated than the forbidden Congo red. Most plants were, with an overall average IC_{50} of 5.34 mg/ml, closer to authorized E163 (IC_{50} = 3.27 mg/ml). Furthermore no detrimental influence of any kind could be observed on the cells. These results combined suggest an absence of cytotoxicity and argue for plant extract safety. Of course in order to further demonstrate inocuity of the raw material studied, additional *in vivo* explorations should be carried out.

Regarding *Rubia tinctorum*, our results did not suggest any cytotoxic activity whereas mutagenic potential was previously described for this dye (Yasui & Takeda, 1983; Kawasaki *et al.*, 1992). Further investigations are needed to properly define the safety margin in term of exposure to lucidin and its phytochemical derivatives that are considered responsible for these effects.

V. CONCLUSION

In conclusion, through evaluation of antioxidant and antiproliferative effects of dyeing plants, the present report suggest the interest of using bioactive and safe phyto-preparations. These raw materials may be considered as multi-functionalised bioproducts useful for many areas of applications. The most obvious one is a come back to their original and traditional use, natural textile dyeing, with potential improvement of light fastness based on self-oxidation control. Moreover, advanced concept of use could lead to formulation of natural textile dye with pharmacological properties (anti-fungal, antibacterial...) that would contribute to the development of functionalised dyed fibres to promote health textile with high value.

Furthermore, it should be noticed that such pharmacological agents with powerful antioxidative property from plant sources are now recognised for their potential interest as therapeutics for pathologies with intense oxidative assault such as diabetes, cardiovascular disease or cancer (Ratnam *et al.*, 2006). The reason behind this lays in that plant active principles with radical scavenging properties are involved in efficiently limiting endogenous molecules glycation and lipoperoxidation phenomenon responsible for cell and tissue

damage. Therefore, in the scope of multi-functionalised natural bio-product our results encourage uses and development of these raw materials for investigation in others areas in addition to dyeing purposes, such as pharmaceutical, cosmetic and agroalimentary. In any case, tested extracts appearing as bioactive potentially non toxic agents, this evaluation needs to be extend in order to assess the absence of toxicity of plant extracts when used in industry (for worker health) and when formulated (for consumer health). This kind of evaluation would probably sustains the potential of natural dyes to be real alternative to synthetic dyes and is of real importance with implementation of the REACH European directive concerning chemicals.

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