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Camille Dubois, Hortense Plainfossé, Maïlys Delcroix, Manon Trinel, Grégory Verger-Dubois, et al.. Anti-Aging Potential of a *Rosa centifolia* Stem Extract with Focus on Phytochemical Composition by Bioguided Fractionation. *Chemistry and Biodiversity*, 2022, 19 (7), pp.e202200158. 10.1002/cbdv.202200158 . hal-04583141

HAL Id: hal-04583141

<https://hal.science/hal-04583141v1>

Submitted on 22 May 2024

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Accepted Article

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This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Chem. Biodiversity* **2022**, e202200158

Link to VoR: <https://doi.org/10.1002/cbdv.202200158>

Anti-aging potential of a *Rosa centifolia* stem extract with focus on phytochemical composition by bioguided fractionation

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Abstract

Agricultural practices generate huge amounts of by-products, often simply discarded as waste that must be processed at some cost. The natural by-products revalorisation as raw material to produce high-added value ingredients for various industrial sectors may pave the way towards more sustainable industrial practices, *via* an optimised utilisation of natural resources. Integrating the circular economy precepts to production systems is considered to be a more and more promising management solution to significantly reduce the environmental impact of economic activities. This article discusses the valorisation of *Rosa centifolia* stem to produce a natural extract with cosmetic anti-aging potential. To do so, the cosmetic potential of 30 extracts obtained by maceration of agricultural by-products in a-hydroalcoholic solvent was evaluated: their activities, as well as their inhibitory activities of specific enzymes were assessed *in vitro* to identify those that could be used effectively as anti-ageing actives while meeting the consumer's expectations in terms of sustainability, naturality, transparency and traceability^[1]. A hydroalcoholic extract of *R. centifolia* stem revealed itself particularly promising

due to its valuable anti-hyaluronidase and antioxidant activities, and its interesting anti-elastase and anti-inflammatory potential. The bio-guided fractionation of this extract allows the characterisation of three major compounds, e.g., isoquercitrin, quercitrin and euscaphic acid, never identified in *R. centifolia* previously.

Keywords: *Rosa centifolia* L. stem; chemical composition; bioguided fractionation; anti-aging potential; up-cycling

1. Introduction

The genus *Rosa* is a large taxon consisting of many species and thousands of cultivars, particularly due to extensive spontaneous hybridization that generates new speciation^[2,3]. The subgenus *Rosa* comprises about 180 species characterised by a variety of colours and scents^[3,4]. These woody perennials are cultivated for multiple purposes, such as cut flowers, outdoor and indoor plants, and fragrancng and flavouring in many industries (perfume, personal care and food sectors mainly)^[5]. *Rosa centifolia* and *Rosa damascena* are the most common roses used in perfumery^[5].

Rosa centifolia, also known as rose de Grasse, cabbage rose or rose de Mai, the "hundred-leaf" rose (*centifolia*) is a complex hybrid rose, which parentage includes *Rosa* × *damascena*. *R. centifolia* plants are shrubs 1.2–1.5 m tall, with flowers characterised by numerous thin, overlapping, highly scented petals, generally light pink, more rarely white to purple. Cultivated throughout the world as a perfume plant, it is particularly grown in the south of France, notably around the city of Grasse known as the capital of perfume, for its odorant qualities. The *R. centifolia* fragrance, often described as clear and sweet, with slight notes of honey, is widely used in the perfume industry, and in personal care products under several forms, i.e. rose water, concrete and absolute^[6]. To do so, *R. centifolia*'s globular flowers, which only bloom for a few days in May, are carefully hand-picked in the early morning and distilled (production of floral water) or extracted while still fresh.

Flowering plants' pruning constitutes a crucial step in flower production to increase the overall performance of the plant as it promotes the plants' growth and aesthetic value (increased flower size, improved flower quality and colour, etc.^[4]). Pruning is usually carried out for multiple purposes: annual pruning improves the plant's health and lifespan, but it is also allows to remove dead wood or to deadhead the plant^[7]. *R. centifolia* is generally pruned during the winter months, i.e. from the second week of December, to the second week of February: downward slanted cuts are usually made no more than 5 mm above the bud^[7]. Poorly flowering stems and old wood are cleanly removed with sharp secateurs or a pruning saw to encourage vigorous new growth^[7]. This practice therefore generates lots of green waste, particularly in the vicinity of Grasse and its well-developed perfume industry, and the large rose-growing fields that result from it. This waste is generally burnt, disposed to landfills or left at the base of the rose shrubs as composting material. While waste incineration and disposal in landfills are the most common waste management solutions, there is growing environmental concern about the unavoidable emissions of harmful gases (such as methane, a potent greenhouse gas) and particulate matter they are responsible of^[8]. As the amounts of such green waste accumulate worldwide, the implementation of environmentally friendly strategies of waste management is mandatory. Composting constitutes such a solution, but will certainly not address waste-related issues^[9]. Including the principles of circular economy in waste management could make a positive difference to the environment.

Such green waste still holds great value, and should rather be considered as raw materials which, after appropriate processing, could lead to the design of active ingredients used in multiple application sectors as nutraceutical and cosmetic. Such an upcycling practice constitutes both a waste management strategy in line with the Waste Framework Directive 2008/98/EC^[10] and responds to growing desire of consumers for more naturality. In fact, concerned and distrustful consumers prioritise natural products over synthetic, including food and cosmetics.

Indeed, this green trend gains ground in the industry, and more particularly the cosmetic ingredients sector. The perpetual ageing of the world population is responsible for the

hegemony of the ever-popular anti-ageing topic: far from being an obsolete concept, a recent study stated that the global anti-ageing market valued at USD 17.28 billion in 2019, was expected to reach USD 27.29 billion by 2026, at a CAGR (compound annual growth rate) of 5.9% over this period^[11].

Skin constitutes the largest organ in the body, and is inevitably submitted to ageing mechanisms under the influence of both intrinsic (chronological ageing, genetics, etc.) and extrinsic factors (environmental for example)^[12,13]. Always generated by normal cellular processes, Reactive Oxygen Species (ROS) can actually be over-produced in response to environmental stress, thus contributing to skin ageing^[14], *via*, notably, activation of enzymes such as hyaluronidases, collagenases and elastases which deteriorate specifically molecules that are responsible for the skin's hydration, or its mechanical properties^[15]. Ageing skin is characterised by laxity, dullness, wrinkles, irregularities, etc.

This article describes the assessment of the marketable potential of agricultural by-products and notably of pruning residues to develop natural cosmetic ingredients that can efficiently impact the skin aging process.

2. Results and Discussion

2.1. Hydroalcoholic *R. centifolia* stem' extract

Several agricultural by-products were selected based on their fundamental nature, e.g., waste, and their accessibility (easily obtainable by-product during the overall duration of the plant cultivation period) to evaluate their potential upgrading as raw materials for the development of natural cosmetic actives. The extraction of these by-products was achieved by maceration in EtOH/H₂O 50/50 v/v, a solvent mixture that was selected as the suitable solvent mixture for the extraction of the compounds of cosmetic interest^[16]. This selection may not be the most appropriate one for each plant by-product but adjustments in solvent or solvents' mixture selection are performed in a second time, once the cosmetic potential of an individual by-product has been evidenced.

The lightening, antioxidant, anti-inflammatory and anti-aging (anti-collagenase, anti-hyaluronidase, and anti-elastase) properties of about thirty extracts were evaluated using *in vitro* assays, and directly confronted to commercially available cosmetic actives, standardized at the same concentration and one appropriate positive standard with a pure molecule (Table 1).

The hydroalcoholic extract obtained from a 2h-maceration of *R. centifolia* stem in EtOH/H₂O 50/50 v/v (rose stem shavings/solvent ratio 1/5 w/w) with an extraction yield of $3.1 \pm 0.2\%$ (extraction performed in triplicate) displays very promising anti-hyaluronidase ($100,0 \pm 3,3 \%$) and antioxidant activities ($92,7 \pm 0,0 \%$), valuable anti-elastase ($60,75 \pm 0,0 \%$), and anti-inflammatory activities ($59,17 \pm 0,0 \%$), and to a lesser extent an interesting anti-collagenase activity ($30,28 \pm 5,1 \%$) (Figure S1).

The HPLC-DAD/ELSD analysis of this extract (Figure S2) indicated a high content in mainly polar molecules (group 1) at retention times lower than 8 min. The HPLC profile also revealed the presence, in smaller quantities, of polyphenolic compounds (group B) eluting between 8 and 40 min and of some apolar molecules eluting after 42 min (group C).

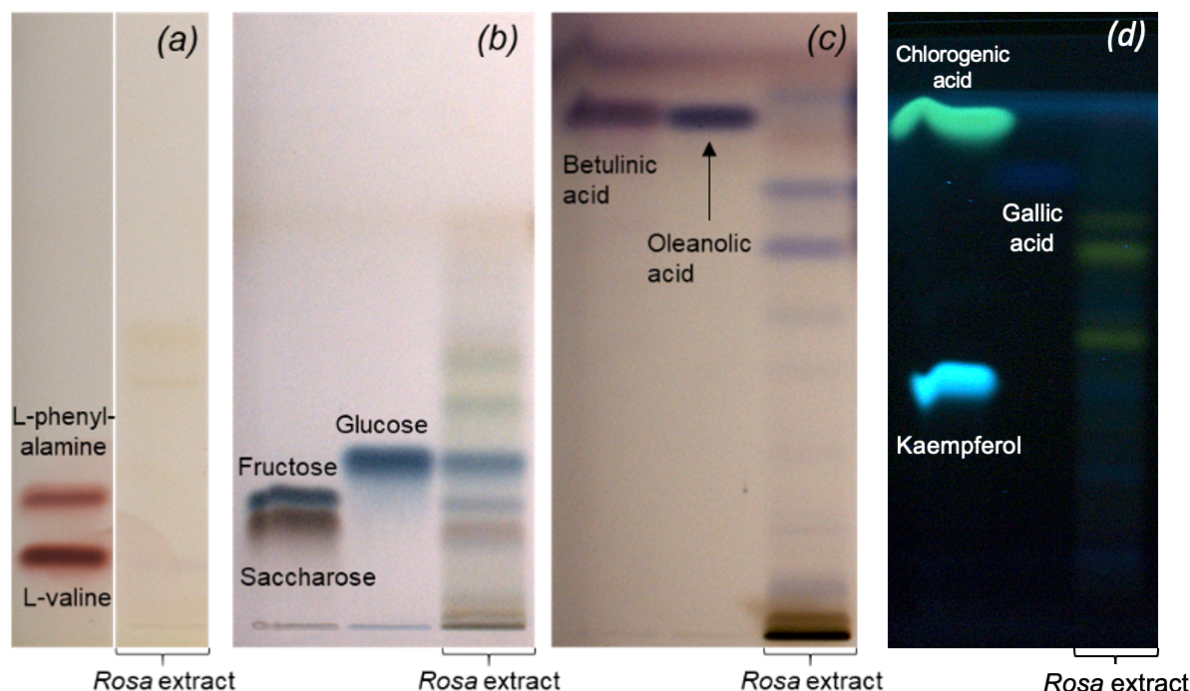


Figure 1. HPTLC plates observed under visible light and UV-366 nm to visualize amino acids (a), sugars (b), less polar compounds (c) and polyphenolic compounds (d) present in the rose stem' extract using ninhydrin, aniline diphenylamine *o*-phosphoric acid and anisaldehyde sulfuric acid mixture, NP and PEG as derivatisation reagents, respectively.

HPTLC analyses provided information on the phytochemical composition to get an overview of the constituents of these groups of compounds (Figure 1).

Concerning molecules of group A, some amino acids could be evidenced using ninhydrin as reagent, but no further characterisation was carried out given the very low intensities of the corresponding bands (Figure 1a). The presence of sugars was assessed using aniline diphenylamine *o*-phosphoric acid as post-chromatographic derivatisation reagent that enables both sensitive and selective identification of sugars thanks to its low detectability, and their colorimetric differentiation (Figure 1b)^[17,18]. Saccharose (dark blue band), fructose (brown band) and glucose (blue band) were therefore identified in this extract by comparison with the corresponding standards^[19].

The presence of less polar compounds (group C) was investigated using anisaldehyde sulfuric acid as derivatisation reagent to assess the occurrence of monoterpenes (blue bands) and triterpenes (purple bands) under white light (Figure 1c)^[20].

Polyphenolic compounds were evidenced in group B using NP and PEG reagents^[21–23]. The colorations of the bands revealed at 366 nm indicate the presence of flavones and/or flavonols (green bands), as well as of phenolic acids and/or coumarins (blue bands, Figure 1d).

2.2. Fractionation of the *R. centifolia* stem' extract

The hydroalcoholic extract obtained by maceration of *R. centifolia* stem was then fractionated several times and the bioactivities of the resulting fractions and sub-fractions were evaluated under the same experimental conditions to establish links between families of molecules and bioactivities.

Reverse phase fractionation of the bulk extract led to the recovery of fractions 1 to 3 that were analysed by HPLC-DAD/ELSD. As evidenced in Figure S3, compounds from group A are predominantly recovered in fraction 1. Fraction 2 appears to enclose the remaining compounds from group A, as well as molecules from groups B and C. Fraction 3 appears to consist primarily of compounds from group C.

The bioactivities of these three fractions were then evaluated in the same experimental conditions as the bulk extract. At the same time, fractions 1 and 2 were analysed by HPTLC; such analysis could not be performed for fraction 3 given the small amount of fraction recovered (recovery yield <1%).

Fraction 1 appears to consist in small amounts of amino acids (Figure 2a) and sugars (Figure 2b) including the three major ones already identified in the bulk extract, i.e., fructose, glucose and saccharose. As shown in Figure 3 this fraction does not show less bioactivity, which allows us to conclude that the amino acids and sugars constituting this fraction are not responsible for the activities evidenced for the bulk extract.

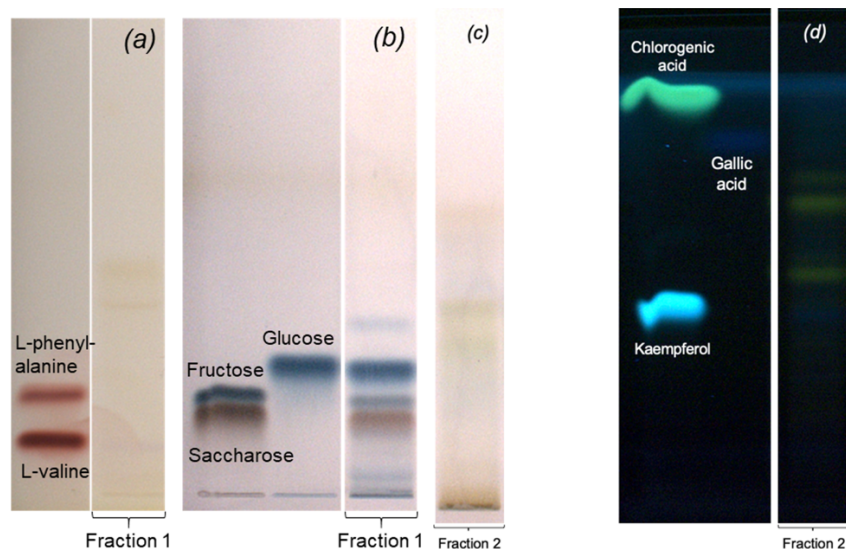


Figure 2. HPTLC plates observed under visible light to visualize amino acids (a) in fraction 1, sugars (b) and (c) present in fraction 1 and 2, and under UV-366 nm to visualize polyphenolic compounds (d) present in fraction 2 using ninhydrin (a), aniline diphenylamine *o*-phosphoric acid (b) and (c) and Neu/PEG reagent as derivatisation reagents (d), respectively.

The HPTLC analysis of fraction 2 revealed the presence of polyphenols that more than likely correspond to the group B molecules, as well as of terpenoids and fatty acids probably belonging to the group C, that are similar to those identified in the bulk extract. Some additional sugars, constituting the remaining group A molecules, were also identified in fraction 2: the colours of the corresponding bands indicate that some compounds are more concentrated than those previously observed. They appear more clearly than in the bulk extract and in fraction 1 (Figure 2). This fraction displays bioactivities quite like those shown for the bulk extract, such as very promising, anti-hyaluronidase and antioxidant activities, as well as moderate anti-elastase and anti-collagenase activities (Figure 3). This fraction even displays better anti-inflammatory activity compared to the *R. centifolia* stem extract: this observation could be linked to the concentration of molecules of groups B and C that occurred in this fraction due to the removal of most of the inactive compounds of group A.

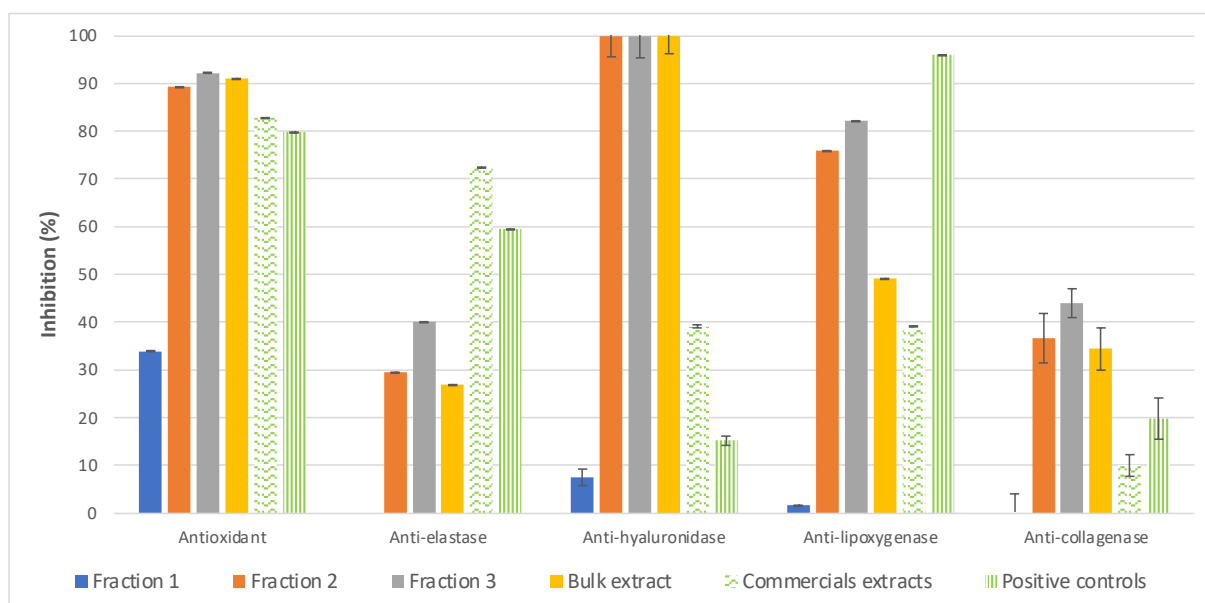


Figure 3. Bioactivities of fractions 1, 2 and 3 obtained by the first fractionation, and comparison with those of the bulk extract. All values are considered with mean \pm SEM, done in triplicate.

Fraction 3 shows similar anti-hyaluronidase, better anti-inflammatory, anti-elastase, anti-collagenase, and antioxidant bioactivities than bulk extract (Figure 3). Its analysis was not carried out due to its low recovery yield, not compatible with a future industrial application. However, given these results, one can conclude that the two groups of molecules B and C are responsible for the bioactivities evidenced.

Given its recovery yield and bioactivities, the analysis of fraction 2 was pursued: this fraction was further fractionated in normal phase to further characterize its phytochemistry. The resulting subfractions were then analysed by HPLC, and their bioactivities were then evaluated as previously.

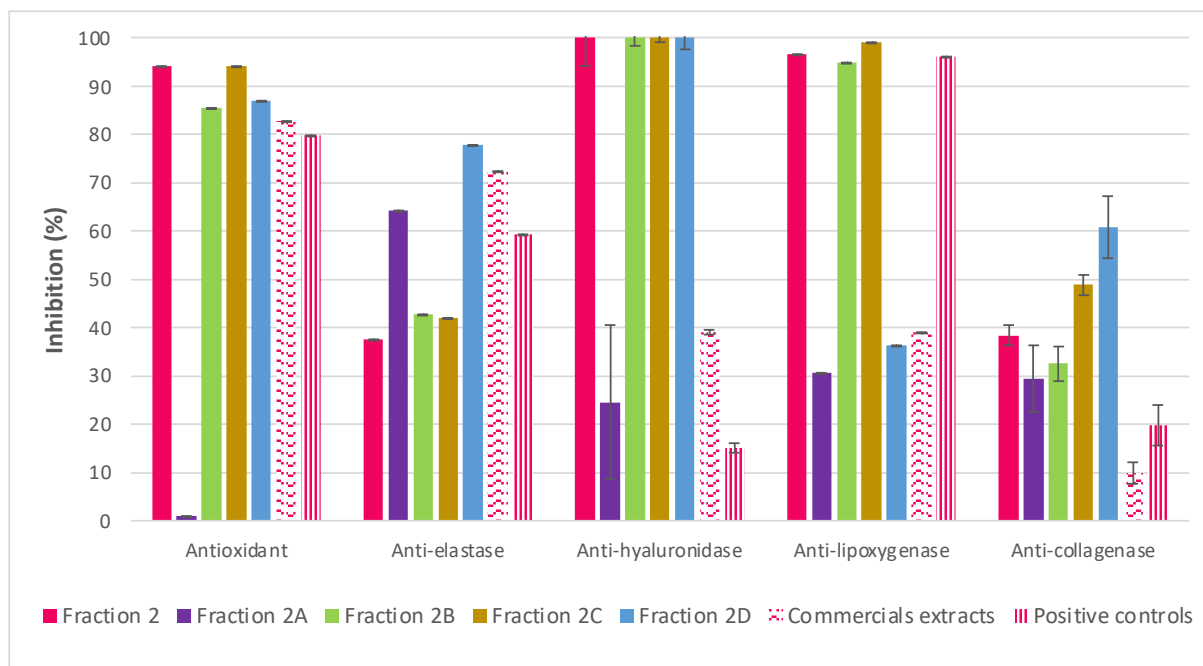


Figure 4. Bioactivities of subfractions 2A to 2D, compared to the bioactivities of fraction 2. All values are mean \pm SEM, done in triplicate.

As shown in Figure S4, subfraction 2A, obtained with a recovery yield of 4.3%, is enriched in compounds of group C: this subfraction mainly displays anti-elastase potential (Figure 4).

The HPLC/ELSD analysis of subfraction 2B recovered with the highest yield (68.3%), indicates that this subfraction is similar to fraction 2: it encloses molecules from groups A, B and C and displays very similar activities to the ones evidenced for fraction 2.

Subfractions 2C and 2D, recovered respectively with yields of 17.6 and 9.8%, are almost exclusively composed of group A compounds, and present interesting bioactivities, quite similar to the ones of the crude extract. This last observation is contradictory to the previous conclusions made on the non-contribution of group A molecules to the bioactivities of the crude extract. In fact, this hypothesis must be qualified according to the chemical composition of the 2C and 2D sub-fractions and the crude extract, which are different. Indeed, the comparative analysis by HPTLC highlighted the differences in composition of group A in the previous fraction 1, and the sub-fractions 2C and 2D (Figure 5).

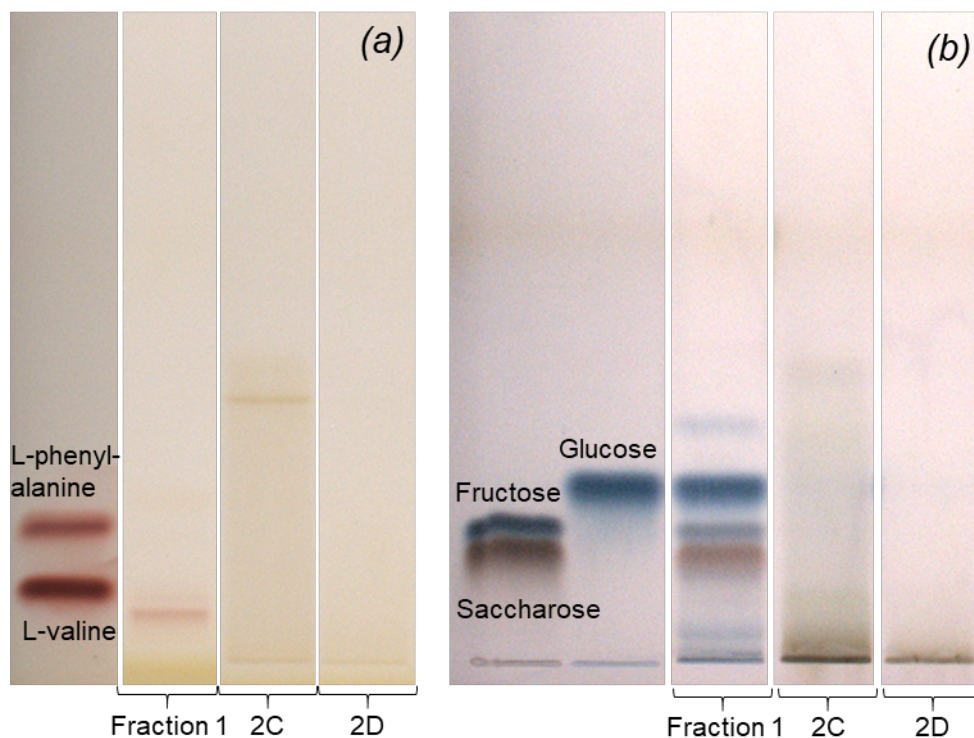


Figure 5. HPTLC plates observed under visible light to visualize amino acids (a) and sugars (b) present in fraction 1 and subfractions 2C and 2D using respectively ninhydrin and aniline diphenylamine o-phosphoric acid as derivatisation reagents.

Subfraction 2B, the most interesting one in terms of bioactivities and recovery yield, was further fractionated in normal phase, to separate the A and B groups of molecules. In doing so, four subfractions were recovered: the polar compounds constituting group A of molecules elute in subfractions 2B-2 (obtained with a recovery yield of 37.5%), 2B-3 (recovery yield of 4.8%) and 2B-4 (recovery yield of 3.6%), while molecules from groups B and C are eluting in subfractions 2B-1 (recovery yield of 54.1%) and 2B-2, as evidenced in Figure 6.

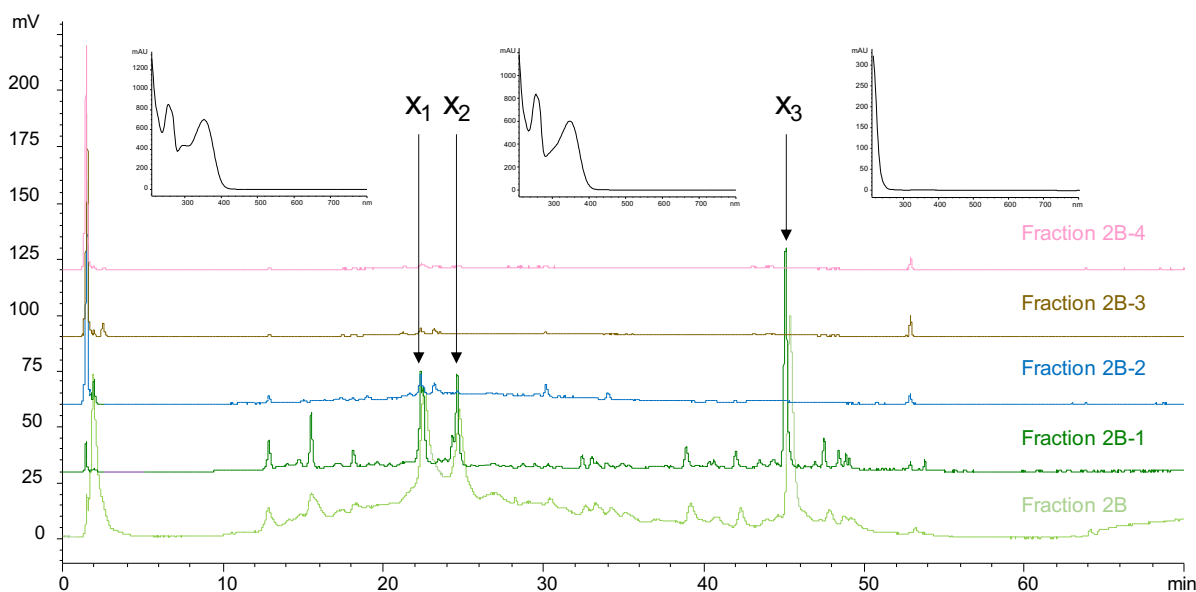


Figure 6. HPLC chromatograms of fraction 2B and of the resulting subfractions 2B-1 to 2B-4 obtained with ELSD, presenting X₁, X₂ and X₃ and their respective UV spectra.

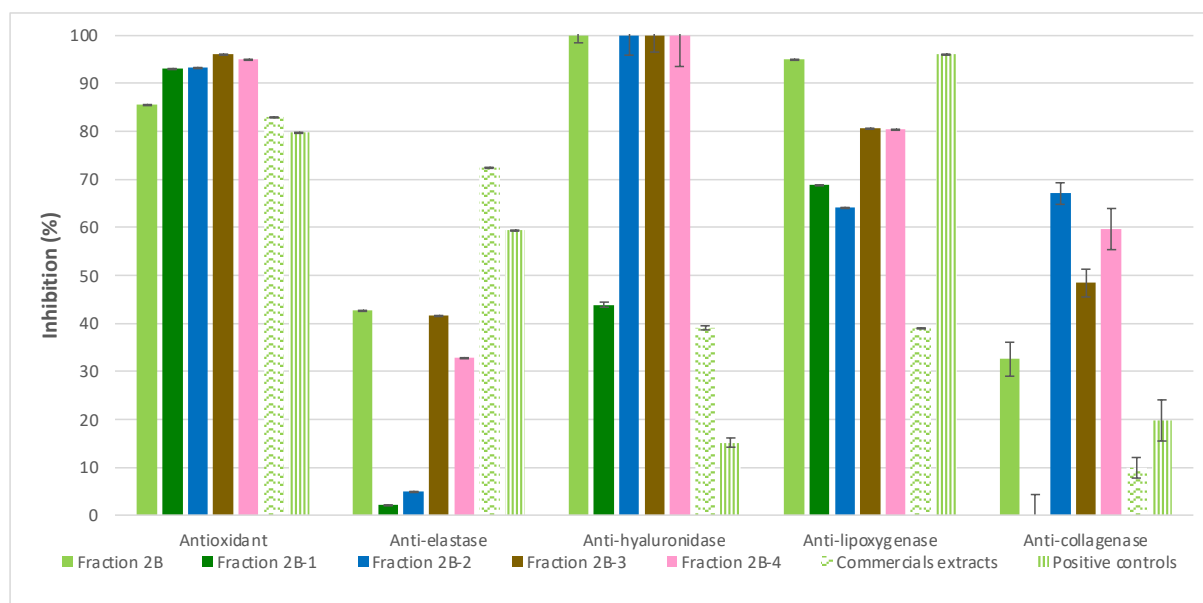


Figure 7. Bioactivities of subfractions 2B-1 to 2B-4, compared to the bioactivities of subfraction 2B. All values are mean \pm SEM, done in triplicate.

Subfractions 2B-1 to 2B-4 all present very promising anti-hyaluronidase and antioxidant activities, similar to or higher than the ones of subfraction 2B. They also display interesting anti-inflammatory and anti-collagenase activities, and subfractions 2B-3 and 2B-4 present some anti-elastase activity. On the contrary, although it shows equivalent anti-inflammatory

and antioxidant activities, as well as some anti-hyaluronidase activity, subfraction 2B-1 does not display any anti-elastase or anti-collagenase activity (Figure 7).

However the analysis of subfractions 2B-3 and 2B-4 was not pursued due to their low recovery yields, and that of subfraction 2B-2 was not pursued either because it still contains a mixture of molecules of the three identified groups.

On the contrary, recovered with an excellent yield and composed of molecules of groups B and C only, the analysis of subfraction 2B-1 was therefore furthered. The HPLC/ELSD of this subfraction indicated the presence of three well individualised compounds, named respectively X_1 , X_2 and X_3 , already present in subfraction 2B. The UV spectra of these three were compared to those of standards available in the laboratory: X_1 and X_2 seem to correspond to flavonols and X_3 to a fatty acid or a lactone.

3.3. Compounds isolation and characterisation

Subfraction 2B-1 was fractionated over silica gel in normal phase using a solvent gradient of increasing polarity; the presence of molecules in each subfraction collected was monitored by TLC using vanillin as visualising agent.

As evidenced in Figure S5, subfraction 2B-1A collected with a recovery yield of 5.6%, appears to be of no interest as it does not contain any of the compounds X_1 , X_2 or X_3 , so its analysis was not pursued.

Subfraction 2B-1B collected with a recovery yield of 16.5%, contains X_3 but the low yield with which this fraction was recovered prevented the isolation of X_3 by semi-preparative HPLC.

On the contrary, subfraction 2B-1C was collected with a recovery yield of 56.6%, an amount compatible with the isolation of X_1 and X_2 by semi-preparative HPLC. Some 47 injections of subfraction 2B-1C resulted in the isolation of 4 mg of X_1 and 5 mg of X_2 .

Compound X_1 is characterised by a protonated ion m/z 465.1016 $[M+H]^+$ associated with the formula $C_{21}H_{20}O_{12}$. A neutral loss of 162 is observed and corresponds to a hexose moiety. The remaining moiety associated with the formula $C_{15}H_{10}O_7$ corresponds to an aglycone moiety

(fragment ion of m/z 303.0491– ESI⁺). The two fragment ions at m/z 151 and 179 (ESI⁻) corresponds to the fragmentation of this aglycone; they are specific to quercetin^[24]. This identification was confirmed by the UV spectrum of the compound X₁. The experimental NMR data compared to those available in the literature allow to characterise it as quercetin-3-glucoside, also named isoquercitrin^[25,26]. This flavonol has never been identified in *R. centifolia*, but it has been described as the main compound in stem of *R. sempervirens*, *R. moschata* and *R. canina*^[27]. It is known to exhibit antioxidant^[28] and anti-inflammatory activities^[29].

Compound X₂ is characterised by a protonated ion m/z 449.1067 [M+H]⁺ associated with the formula C₂₁H₂₀O₁₂. A neutral loss of 146 is observed and corresponds to a rhamnose moiety. The remaining moiety associated with the formula C₁₅H₁₀O₇ corresponds to an aglycone moiety (fragment ion of m/z 303.0491– ESI⁺). This aglycone moiety was identified as quercetin, as evidenced by the occurrence of the same fragment ions as previously discussed for compound X₁. By comparing the experimental NMR data obtained for this compound with data available in the literature, X₂ was identified as quercetin-3-rhamnoside, also known as quercitrin^[30]. Never identified in *R. centifolia*, it was previously described in an extract from the fruit of *R. laevigata* Michx^[31] and in extracts of *R. canina*, *R. corymbifera*, *R. micrantha* and *R. sempervirens* rosehips^[32]. The bioactivities of compound X₂ were reported in the literature^[28,30], this molecule exhibits strong anti-hyaluronidase and antioxidant activities.

Subfraction 2B-1B, mainly composed by compound X₃, was analysed by UPLC-HRMS. Compound X₃ has a theoretical mass m/z 489.3568 [M+H]⁺ with the corresponding formula C₃₀H₄₈O₅. According to Chen Jian et al., who identified euscaphic acid in dried leaves of a Rosaceae Asian shrub, the NMR spectrum described is similar to the compound X₃^[33]. The NMR analyses of the subfraction allowed the identification of compound X₃ as euscaphic acid, a pentacyclic triterpene already evidenced in many *Rosa* species^[34–36], but never in *R. centifolia*.

Its anti-inflammatory^[34,37] and antioxidant activities^[38] were already reported in the literature.

Praised for their beauty and aroma, roses are the most widely grown flowers in the world, especially as ornaments (cut flowers, plants) and for the perfume industry. The cultivation of roses is therefore a profitable activity, and the importance of this market implies inherent large amounts of waste generated during cultivation. The revalorisation of pruning waste to produce high added value ingredients for several sectors such as cosmetics might generate new income for farmers, while freeing them from the costs of waste management.

Such *R. centifolia* stem extract was therefore found to be a good candidate to develop a new anti-aging cosmetic active, provided that some adjustments of the extraction conditions are undertaken to improve the extraction yield and be more economically viable, while preserving the bioactivities evidenced. Such attempts, including varying the hydroalcoholic ratio, replacing the solvent with propylene glycol, etc., have already been undertaken but have been unsuccessful so far; extraction assistance by activation technologies might be the key to improve this extraction yield.

The steps required to develop an active are numerous (Figure S5): natural extracts are generally not added as is in cosmetics and studies on solubility, discolouration, deodorization, and stability must be done before formulation. The addition of an appropriate cosmetic carrier can facilitate the incorporation of extracts into cosmetics formulation.

3. Conclusions

In this article, the potential of pruning residues as a raw material to develop an anti-age active is evaluated. Three major compounds were isolated: isoquercitrin, quercitrin and euscaphic acid. They are partly responsible for the cosmetic activities evidenced for the hydroalcoholic extract of *R. centifolia* stem. This extract must be improved, and various extractions techniques will notably be tested to increase its recovery yield. Once the ingredient is developed and the active molecules are characterised, quality, efficacy, shelf life and safety/tolerability tests must be performed before the commercial launch of the product. Toxicological evaluation is mandatory in order to comply with the guidelines published by the Scientific Committee on

Consumer Safety (SCCS) at an European level^[39], while worldwide, the U.S. Food and Drug Administration (FDA) guarantees the application of this assessment.

To avoid the unpleasant discovery of toxicity problems, it is recommended to perform a minimum of tests such as ocular or skin irritability, skin sensitization, phototoxicity and mutagenicity, carcinogenicity, reprotoxicity assays during the R&D process. To complete all the toxicity files, analyses of pesticides and/or contaminants (herbicides, pollutants) can be undertaken on the final cosmetic ingredient, even if no pesticides were used on the raw material.

The development of new natural ingredients intended for use in cosmetics is a time-consuming process which can normally take several years. In fact, the raw material needs in most of case new cultivation, and this step can take several years (2-3 years). However, the upcycling materials are ready to use. In this case, the time-savings is very important for the development of new cosmetic ingredient.

4. Experimental Section

All chemicals were obtained from Sigma-Aldrich (Saint-Louis, USA) unless otherwise stated.

4.1. Plant material

R. centifolia stems were obtained from the Rosessences domain, located in Grasse, France. Pruned at the end of January and beginning of February 2020, stems were sorted during collection to eliminate deadwood, left to dry in the ambient air sheltered from the rain and crushed into tiny shavings until extraction. A voucher specimen of *R. centifolia* was deposited and identified by Olivier Gerriet, in the herbarium collection of the Natural History Museum of Nice, France, under the reference number NICE-D-5931.

The extraction was performed by direct maceration and more detailed in supporting information.

4.2. High Performance Liquid Chromatography

The crude extract, fractions and subfractions diluted at 10 mg/mL in methanol (MeOH; chromatography grade) and filtrated over 0.2 µm PVDF (polyvinylidene difluoride) syringe filter, were analysed using an Agilent 1200 HPLC system (Courtaboeuf, France) equipped with a DAD (Diode Array Detector) and an ELSD (Evaporative Light Scattering Detector) operating under the following conditions: injection volume: 20 µL, and flow rate: 1.0 mL/min. Separations were performed on a C18 column (Phenomenex, Le Pecq, Ile-de-France, France; Luna® 5 µm, 150 mm × 4.6 mm i.d.).

4.4. High Performance Thin-Layer Chromatography

High performance thin-layer chromatography (HPTLC) was performed on 10 cm x 20 cm HPTLC silica gel 60 F254 pre-coated plates (Merck, Darmstadt, Germany). Two microliters of each standard and sample were applied to the plate using an automated ATS4 sampler (Camag, Muttenz, Switzerland). Separation on the plate was performed in an automatic ADC2 developing chamber (Camag, Muttenz, Switzerland). After development and drying, the plates were immersed in the detection reagent using the Camag immersion device, dried in a stream of warm air and immersed in a specific revelation reagent. The bands were visualised under visible and UV (366 nm) light using the Camag TLC Visualizer.

The solvent systems and revelation reagents, chosen according to the phytochemicals analysed, are described in supporting information.

4.5. Bioassays

Bioassays were performed as presented previously in untreated 96-well plates (Thermo Nunc, Villebon-sur-Yvette, France), apart from the lipoxygenase assay which was performed in UV-transparent ones (Costar-Merck, Darmstadt, Germany) ^[1,16]. Plates were sealed during incubation periods with adhesive films (Greiner Bio-One, Courtaboeuf, France). Samples, e.g., crude extract, fractions, isolated molecules and controls, were diluted to a concentration of 3.433 mg/mL (final concentration) in dimethyl sulfoxide (DMSO) in 1.5 mL Eppendorf tubes,

suitable for use with the epMotion® 5075 automated pipetting system (Eppendorf, Montesson, France). The activities of all samples were evaluated in triplicate.

Table 1. Positive controls used in each bioassay performed.

Assay	Commercials extracts (3.433 mg/mL in DMSO)	Positive controls
DPPH assay	<i>Camellia sinensis</i> commercial extract	Resveratrol (3.433 mg/mL in DMSO)
Lipoxygenase assay	<i>Arnica montana</i> L. commercial extract	Resveratrol (3.433 mg/mL in DMSO)
Elastase assay	<i>Rubus idaeus</i> L. commercial extract	Quercetin (2.59 mg/mL in DMSO)
Hyaluronidase assay	<i>Rubus idaeus</i> L. commercial extract	Resveratrol (3.433 mg/mL in DMSO)
Collagenase assay	<i>Rubus idaeus</i> L. commercial extract	Tannic acid (2.93 mg/mL in DMSO)

4.6. Fractionation of the rose extract compound isolation

The EtOH/H₂O 50/50 extract of *R. centifolia* was then fractionated to identify the molecules or families of molecules responsible for the activities evidenced according to the procedure described in Figure 8. More details about the fractionation procedure are in supporting information.

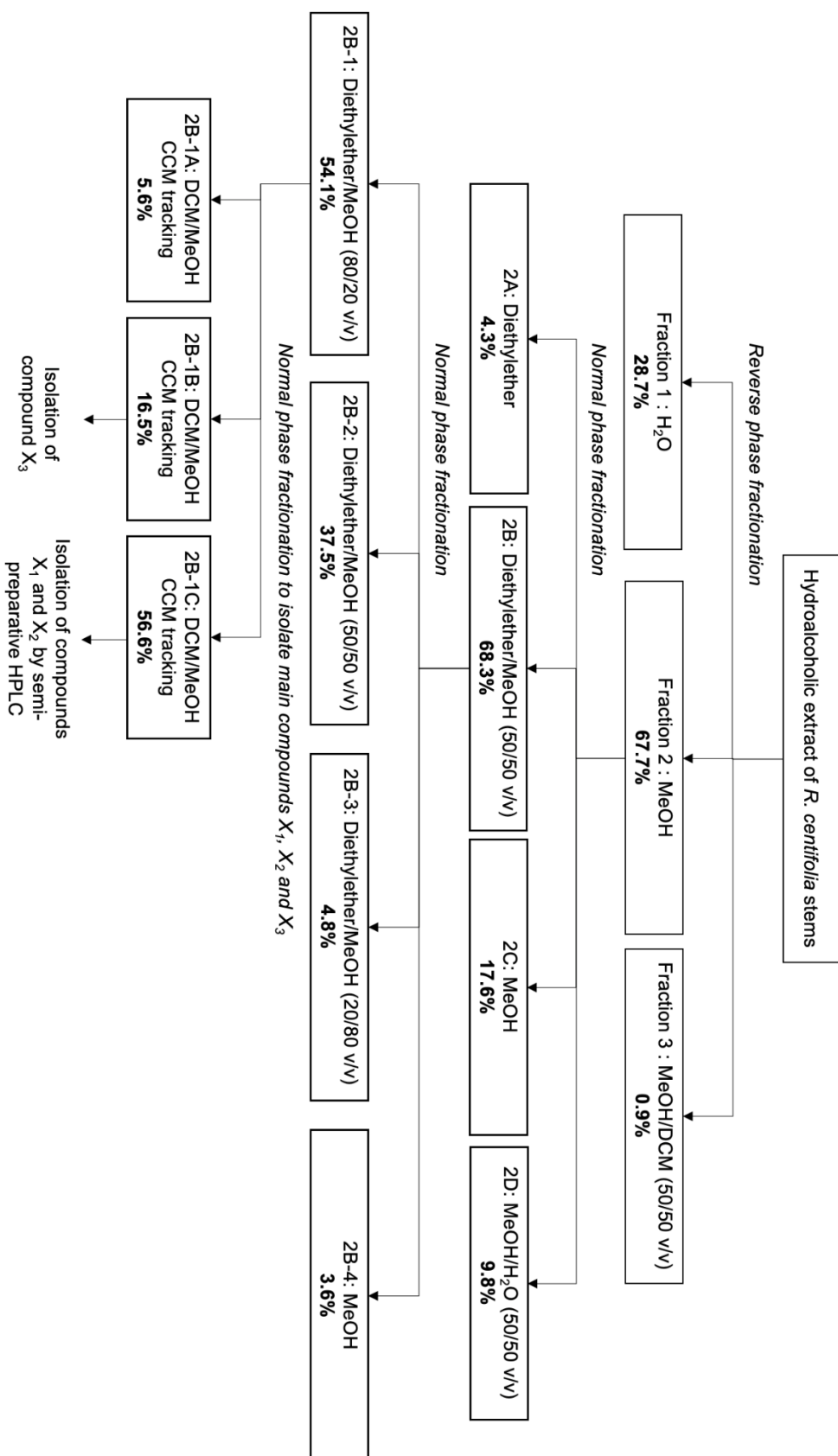


Figure 8. Fractionation scheme of the crude extract of *R. centifolia* stem.

4.8. Ultra-High-Performance Liquid Chromatography–High Resolution Electrospray Ionization Mass Spectrometry

Fractions containing compounds X₁, X₂ and X₃ were then analysed by Ultra-High-Performance Liquid Chromatography–High Resolution Electrospray Ionization Mass Spectrometry. All data are given in supporting information.

4.9. NMR

1D (¹H, ¹³C) and 2D (COSY, HSQC, HMBC) NMR spectra were recorded for compounds X₁, X₂ and X₃ in CDCl₃, at 25 °C on a 400 MHz Bruker® Avance NMR spectrometer. All data are given in supporting information.

Funding sources

This research project was financially supported by the French ANRT - Association Nationale de la Recherche et de la Technologie, grant number 0036/2016.

Acknowledgments

The authors wish to thank François Oulmont (Rosessence, Grasse) for kindly giving us access to the *Rosa centifolia* by-products and Olivier Gerriet from the Natural History Museum of Nice for the realization of the rose deposit.

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Graphical abstract

