Wastewater to Ecological dyeing process and bioactive compounds resources: case study of Dittrichia graveolens hydrodistillation aqueous residue

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Research

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Abstract

The release of aqueous residues generated by the extraction process of essential oils presents a real risk of environmental pollution. This work aims to reduce this risk and produce value-added materials. The aqueous residue of *Dittrichia graveolens* (*D. graveolens*) hydrodistillation has been reused in two valorization ways: 1/ in the ecological dyeing. 2/ in biological field. First, a phytochemical study of the aqueous residue was carried out by determining the ploy phenols and flavonoids content and high performance liquid chromatography (HPLC) analysis. Second, the eco-dyeing process with the aqueous residue was performed on the polyamide fabric, the process was optimized by the surface response methodology using Minitab software and was evaluated by the fastness tests for the optimal conditions. Third, the aqueous residue was assessed for its biological activities. Promising coloring power and biological potential of the aqueous residue showed that this last could be an important source for developing environmentally friendly natural dyes and bioactive products.

Background

The textile dyeing and the essential oil extraction industries are among the most successful industries in the Mediterranean basin (Plan, 2002; Baser and Buchbauer, 2015). Although their fields of activity are not related, these two types of industries have in common, in addition to their geographical proximity, their considerable volume of colored water discharges. Indeed, the amount of water generated by dyeing one kilogram of fabric is about 60 L, depending on the dyeing process (Bhatt and Rani, 2013). This water, strongly colored, represents a real ecological disaster that pushes more and more "eco-conscious" consumers to boycott textile products containing synthetic dyes. The same is true for buyers of essential oils who are generally health conscious and who realize that the product they have purchased is obtained through a non-ecological process. Indeed, the hydrodistillation extraction of one milliliter of oil requires the consumption of 0.5 to 1 liter of water (Wu et al., 2015; Filly et al., 2016; Gharred et al., 2019). At the end of the extraction process, the colored residual water is totally discharged in the environment without appropriate color removal treatment.

In the face of this ecological awareness that characterizes the first half of the 21st century, scientific research has increasingly focused on the development of cleaner industrial processes. Thus, in the field of textile dyeing, researchers such as Ben Ticha et al. have conducted extensive works to replace synthetic dyes, environmentally toxic, with natural dyes while achieving excellent dyeing performance (Ben Ticha et al., 2016). Other researchers have concentrated their efforts on minimizing water consumption and even replacing it with a so-called green solvent: supercritical CO_2 (Banchero, 2013). Bishr et al. (2018) have extracted a fraction enriched in active principals (γ–Pyrones) from *Ammi visnaga* using supercritical CO_2 as an alternative solvent for methanol, ethanol or hydro-ethanol.

The idea behind this work is to reduce the environmental impact of the essential oil extraction process by two complementary ways of valorisation: textile fiber dyeing by the aqueous residue of the hydrodistillation rich in colored substances and highlight the biological potential of the aqueous extract.

Inula is a vegetal genus that belongs to the *Asteraceae* family. It includes a variety of about 100 species, and is widely distributed in the Mediterranean basin. Species of this genus have been reported in the literature as having ethnopharmacological applications, to treat a wide range of disorders, mainly respiratory, digestive, inflammatory, dermatological, cancer and microbial diseases (Seca et al., 2014) thanks to their secondary metabolites such as flavonoids, sesquiterpenes and essential oils… *Inula graveolens* (L.) Desf. (Synonym: *Dittrichia graveolens* L. Greuter) is an annual aromatic plant with a foul camphor odor, it blooms from June to August. It is a nitrophilic species, growing on cultivated land, abandoned fields, roadsides and rural areas. Its leaves are oval and pointed and its flowers have yellow petals. Several studies concerning *D. graveolens* from different geographic zones have been reported and revealed its important pharmacological effects. Indeed, the *D. graveolens* ethanolic and methanolic extracts from Iraq, Jordan, Tunisia or Turkey showed antioxidant (Al-Fartosy, 2011), antiproliferative (Abu-Dahab and Affifi, 2007), allelopathic, antifungal (Omezzine et al., 2011), cytotoxic and antibacterial (Topçu et al., 1993) activities respectively. Moreover, the *D. graveolens* essential oil thanks to its wide use in aromatherapy was developed on a commercial scale (Blanc et al, 2004) under the name "odorous *Inula*"; this essential oil is recognized for its powerful actions on the respiratory system: mucolytic, expectorant, anticatarrhal and antitussive action and it is also known as a regulator and cardiac tonic. The distillation of the essential oil generates a considerable quantity of colored water discharge and according to the literature, no study has demonstrated the importance of these residues in textile dyeing. We therefore chose to focus for the first time on the reuse in an environmentally friendly way of the aqueous residue from the hydrodistillation of Tunisian "*Inula graveolens*" as a dye bath and evaluated its biological potential.

In this study and first of all, a chemical characterization of the aqueous residue was evaluated. Secondly, the aqueous residue was valorized as a dye bath for the polyamide fabric, the dyeing process was optimized thanks to Minitab 18 software using the response surface methodology (RSM). The color strength parameter (K/S) and the fastness values were determined for the optimum dyeing conditions. Finally, the antioxidant, antibacterial, cytotoxic and anti-inflammatory activities were evaluated in order to estimate the biological potential of the hydrodistillation aqueous residue.

Materials And Methods

The plant

The plant *Dittrichia graveolens* was selected by Dr. Ridha El Mokni, a researcher in botany at the faculty of pharmacy of Monastir, Tunisia. The voucher specimen was deposited at the herbarium (*Asteraceae*, n°23) of horticulture and breeding school of Chott-Meriem (University of center, Sousse, Tunisia). The plant was harvested in November 2015 in an olive grove in Monastir (Tunisia) where it grows wild. The leaves and flowers were separated from the stems to be dried in the dark and at room temperature. The dried leaves and flowers were then milled and used for the remainder of this study.

Aqueous residue preparation

The previously dried and ground leaves and flowers (100 g) were added to distilled water (1 L) and placed in a Clevenger apparatus to hydrodistillate the solid material for 3 hours. The essential oils obtained is the subject of an independent study. The residual mixture (plant material + water) was filtered under vacuum to recover a colored aqueous extract whose concentration is 100 mg of dry matter per mL of water.

Phytochemical study of the aqueous residue of *D. graveolens* hydrodistillation

The total polyphenols were estimated using the Folin-Ciocalteu reagent according to a protocol described previously (Boveiri Dehsheikh et al., 2019). On the other hand, the flavonoid contents were determined by the method developed by Bouzidi et al. (Bouzidi et al., 2016).

The Ultraviolet-visible (UV-vis) spectrum of the aqueous extract was obtained using a Campspec M 108 spectrophotometer.

The HPLC spectra of the aqueous residue were performed using an Agilent 1200 Series HPLC System. The analysis was carried out according to the following protocol (FaríasCampomanes et al., 2015): Initially, 10 µL of the extract to be separated are injected at the inlet of the column (reversed phase C18, 100 × 4.6 mm x 2.6 microns). The mobile phase, consisting of two eluents containing 0.1% acetic acid: phase A (water) and phase B (acetonitrile), flows at high pressure at 2 $mL.min^{-1}$. At the column outlet, the detection of two main phenolic compounds, quercetin and catechin, was carried out using an UV-vis detector set at 254 and 280 nm, respectively. For this purpose, the analytical method by standard addition was used (Pistos et al, 2014).

The Infrared (IR) spectrum of coloring powder obtained after lyophilization of the aqueous *D.graveolens* extract was carried out by a Perkin Elmer FTIR infrared spectrometer.

Dyeing quality evaluation by the aqueous residue

Dyeing protocol with the aqueous residue

The polyamide (jersey and weight of 302 $g.m^{-2}$) was chosen following the results obtained by the preliminary dyeing tests of a multifiber fabric (see Fig. S1). The dyeing process was carried out in a laboratory-dyeing machine (Ahiba Datacolor International, USA) at 60 °C for 60 min with a liquid ratio of 40:1. The dyed fabric was then rinsed with warm water and soaped with a nonionic detergent. It was finally washed again with cold water and dried at room temperature.

Infrared spectroscopy analysis

The IR spectra of the textile fibers before and after the dyeing were carried out using the Perkin Elmer FTIR infrared spectrometer.

Color measurement and fastness testing

The dyeing quality was evaluated using the color strength parameter (K/S) measured by SpectroFlash SF300 spectrophotometer (Datacolor International, USA) using D65 and 10° standard observer. The (K/S) values were calculated at 410 nm using the Kubelka–Munk equation (Haddar et al., 2014):

$$K/S = (1 - R)^2 / 2R\text{-}(1 - R_0)^2 / 2R_0 \quad (1)$$

Where R is the decimal fraction of the reflectance of the dyed fabric, R_0 is the decimal fraction of the reflectance of the undyed fabric, K is the absorption coefficient and S is the scattering coefficient.

Specific tests include color fastness to washing according to ISO 105-C06, colorfastness to rubbing ISO 105-X12 and colorfastness to light ISO 105-B02.

Experimental design and optimization

Optimisation studies were conducted using response surface methodology (RSM) and Minitab 18 software (Version18, State College, PA, USA). To evaluate the effect of each selected experimental parameter on the results obtained, regression and variance analysis (ANOVA) was used. The experiments were established based on a Central Composite Design Method (CCD) for three factors (temperature, duration and pH) and three levels.

Evaluation of the biochemical potential of the aqueous residue of *D. graveolens* hydrodistillation

The sample performance for all the tests was assessed by performing triplicate assays in the same situation.

Anti-oxidant activity

The antioxidant activity was evaluated by two methods: DPPH (2,2-diphenylpicrylhydrazyl) radical scavenging test which allows the evaluation of the anti-radical capacity of a sample and ORAC (Oxygen Radical Absorbance Capacity) test which measures the capacity of a sample to inhibit the oxidation of a target molecule (fluorescein) induced by a source of radicals.

- Assay of DPPH radical scavenging activity

The antioxidant activity of the aqueous extract of *D. graveolens* was evaluated by the DPPH radical scavenging assay as described by Yu et al., 2008. The inhibition percentage (% IP) of DPPH radicals was calculated by the following formula (Tian et al., 2012):

$\% IP = (Abs_0 - Abs_i) / Abs_0 \times 100$ (2)

Where Abs_0 is the absorbance of the negative control (0.1 mM DPPH solution) at 517 nm and Abs_i is the absorbance, at the same wavelength, of 0.1 mM DPPH solutions containing different concentrations (0 to 0.12 mg. mL^{-1}) of the sample to be tested.

The inhibitory concentration (IC_{50}), which corresponds to the amount of the sample required to remove 50% of the DPPH groups, could thus be determined graphically.

- ORAC test

ORAC test was carried out according to the method developed by Cao et al. (1993) and improved by Ou et al. (2001), using 2,2'-azobis-(2-amidinopropane)-dihydrochloride (AAPH) as a peroxyl radical generator and fluorescein as a fluorescent probe.

AAPH (414 mg) was dissolved in 10 mL of 75 mM Phosphate Buffered Saline (PBS) (pH 7.4); a fluorescein stock solution 4 µM was prepared in 75 mM PBS buffer and kept at 6 °C; this stock solution was diluted 1000 times immediately prior to use. Measurements were performed using a 96-well microplate; 150 µL of the fluorescein solution was added in all experimental wells; 25 µL of sample solutions prepared at different concentrations were added while 25 µL of buffer was used for the blank. After incubation at 37 °C during 30 minutes, the reaction was initiated by the addition of 25 µL of the AAPH solution; the intensity of fluorescence was recorded every 1 min for 2 h at respective excitation and emission wavelengths of 483 and 530 nm, using a microplate reader of BMG LABTECH type, CLARIOstar.

The influence of a given sample on the degradation of fluorescein may be evaluated by measuring the area under the fluorescein quenching curve, with or without antioxidant.

The area under the curve (AUC) was calculated as follows:

$AUC = 1 + (It_1 / It_0) + (It_2 / It_0) + ... + (It_{120} / It_0)$ (3)

Where I is the intensity of the fluorescence, t_0 is the time at 0 min and t_n the time at n min. Interpreting the ORAC analysis data involves calculating the AUC net:

$AUC_{net} = AUC_{sample} - AUC_{blank}$ (4)

Antibacterial activity

Antibacterial activity was evaluated for the aqueous extract against *Vibrio parahaemolyticus* ATCC17802, *Vibrio alginolyticus* ATCC17749, *Staphylococcus epidermidis* CIP3106510 and *Escherichia coli* ATCC35218 using disk-diffusion tests.

Bacterial suspensions (10^6 CFU.mL^{-1}) were inoculated on the surface of Mueller Hinton agar plates. A first test consists of placing 10 µL of the diluted extract (10 mg.mL^{-1}) on filter disks inoculated with Mueller Hinton agar (Biorad, France). The inhibition zone diameters around each disk were measured after 18 hours of incubation at 37 °C. Chloramphenicol solution (Sigma-Aldrich, Switzerland) was used as a reference.

Anti-inflammatory activity

Anti-inflammatory activity was conducted using Swiss mice (20–25 g) of 6–8 weeks old. The animals were treated in accordance with guidelines established by the European Union for the Use and Care of animals (CEC Council 86/609).

The investigation of anti-inflammatory properties was carried out according to the method described by Kou et al. (2005) slightly modified. The mice are divided into several lots, each comprising 6 mices: one batch (negative control) receives nothing, two batches representing positive control (reference): the first receives dexamethasone and the second receives aspegic (15 mg. Kg^{-1}), the last batch receives the extract in different doses (5 and 10 mg. Kg^{-1}).

A thirty minutes period after intraperitoneal administration of the extract or dexamethasone, 30 µL of xylene (phlogogenic agent) were applied to the internal and external surfaces of the right ear of each mouse. The left ear was considered a witness. The thickness of the ear was measured using a digital caliper three hours after the induction of inflammation. The difference in thickness between the two ears was determined. The percentage inhibition of edema compared to the control group was calculated according the following formula:

$Inhibition \% = [1-(\Delta E_t / \Delta E_c)] \times 100$ (5)

Where

E_t is the average edema in the treated groups and E_c is the average edema in the untreated group (control group).

Cytotoxic test

A cell line of skin healthy human fibroblast CCD-45 SK ((ATCC® CRL 1506) was used in order to investigate the cytotoxicity effect of the *D. graveolens* aqueous extract. This test was carried out according to the method described by Noudogbessi et al. (2014), using 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazoliumbromide (MTT) as reagent. This method is based on the capacity cells to reduce MTT to a colored product: formazan. The cells were seeded in a 96-well microplate at the rate of 2,000 cells per well in 150 µL of culture medium, followed by a 24 hours incubation. The cells were then incubated with extract at concentrations between 0.005 and 0.2 mg.mL^{-1}. Briefly, 15 µL was added per well of MTT (0.5 mg.mL^{-1}) followed by

a 4 hours incubation. The appearance of crystals is proportional to the number of living cells. The supernatant was removed, the crystals were dissolved in 150 µL of an ethanol / dimethyl sulfoxide (DMSO) solution (50:50) and its absorbance was measured by a Multiskan microplate reader (Thermo Scientific, Courtaboeuf, France) at 540 nm.

Results And Discussion

Phytochemical study of the hydrodistillation aqueous residue

The aqueous residue of the hydrodistillation of *D. graveolens*, characterized by a yellowish-brown color, has a concentration of total polyphenols and flavonoids of 140 mg.EqGA.g^{-1} extract and 47 mg.EqC.g^{-1} extract, respectively. Fig.A.1 shows the UV-visible and infrared spectra of the aqueous residue which confirm the presence of flavonoids. Indeed, the UV-visible spectrum (Fig. A.1 (a)) has two characteristic absorbance peaks at 290 and 330 nm. The first peak can be attributed to the benzoyl function while the second one is related to the cinnamoylacid form of the molecules (Kheyar-kraouche and Bento, 2018). On the other hand, the infrared spectrum of the aqueous residue (Fig. A.1 (b)) reveals the characteristic bands relating to flavonoids such as: a broad band attributable to the hydroxyl functions at 3400 cm^{-1}.

A more specific exploration of flavonoid content in the aqueous residue was carried out by HPLC using two standards: quercetin and catechin. Indeed, these two molecules are known to be responsible for the yellow color of certain flowers (Bechtold and Mussak, 2009). Thus, Fig. 1 shows the chromatograms of the hydrodistillation aqueous residue in which quercetin and catechin were, respectively, identified. The amount of quercetin and catechin in the aqueous extract was evaluated at 4 mg.g^{-1} of extract and 5.92 mg.g^{-1} of extract respectively.

Textile dyeing using the hydrodistillation aqueous residue

Dyeing of multifiber fabric: tests of validation

The aqueous extract of the mixture of leaves and flowers of *D. graveolens* was tested to dye a piece of multifibre fabric. Fig. A.2 gives the result of this test and shows that brown, light yellow and mustard yellow colorations can be obtained on wool, cellulose acetate and polyamide, respectively. Based on the preliminary tests, further studies are only focused on the polyamide fibers dyeing which gave the deepest shade when dyed with hydrodistillation aqueous residue.

Catechin and quercetin are the two main coloring molecules identified during the chemical characterization of the aqueous extract. In order to validate their contributions in the dyeing process, three synthetic dyebaths were prepared containing a known amount of each of the molecules taken alone and as a mixture. Table 1 shows the results of the polyamide dyeing by the three synthetic dyebaths as well as that performed by the hydrodistillation aqueous residue. When comparing the different shades of color obtained, it seems that catechin and quercetin, present in the aqueous residue of the hydrodistillation of the leaves and flowers of the *D. graveolens* plant, are the main molecules responsible for the dyeing of polyamide fabrics.

Identification of the chemical mechanism of dyeing

Once the coloring molecules identified, it becomes possible to specify the nature of the interaction involved in the dyeing mechanism. Table 2 shows the effect of varying the pH of the aqueous extract of *D. graveolens* hydrodistillation on polyamide fiber dyeing. The results reveal that the aqueous extract loses its coloring power at neutral and basic pH.

The UV spectra of the aqueous extract at different pHs presented in this same Table 2, highlight the effect of pH on the dyeing process. Indeed, the pH increase causes a decrease in the absorbance of the aqueous residue. This observation suggests that hydroxyl groups (OH) of the dye molecules and the amide functions of the fiber are directly involved in the dyeing mechanism, probably through hydrogen bonds.

Modelling and optimization of the dyeing process

The experimental design included fifteen polyamide dyeing experiments, which were conducted according to the scheme mentioned in Table 3.

The performance of the dyeing process of polyamide fabric using the aqueous extract of *D. graveolens* was evaluated by measuring the strength parameter (K/S), which is dependent on the following input factors: the pH of the dye bath (3, 5 and 7), the dyeing temperature (40, 60 and 80 °C) and the dyeing duration (30, 60 and 90 min).

Response surface methodology regression

The matrix design and the corresponding results of RSM experiments were shown in Table 3. The regression analysis of the experimental data displayed that the correlation between the response variables and the test variables was established by the following second-order polynomial equations:

$$K/S = -0.55 + 1.8pH - 0.17T(^\circ C) + 0.071t(min) - 0.13pH * pH + 0.0027T(^\circ C) * T(^\circ C) - 0.00019t(min) * t(min) - 0.0074pH * T(^\circ C) -$$

(6)

Where K/S is the strength parameter, T(°C) is the dyeing temperature and t(min) is the dyeing duration.

The model has a regression coefficient R^2 = 98.98%, which indicates good predictability in the chosen range of variables.

Variance analysis (ANOVA)

A variance analysis was performed to evaluate the main effects of the factors influencing the performance of the dyeing process. Indeed, p values (given in Table A.1) lower than 0.05 indicate that the model and the estimated parameters are statistically significant (Enid et al., 2005). According to Table A.1, the regression model obtained from Eq. (3) is highly significant. Moreover, it seems that the factors pH and temperature are statistically significant followed by the factor duration (p = 0.047). However, the interaction between temperature and duration dyeing is statistically not significant (p = 0.11).

Analysis of the contour plots

The analysis of the effects of the different parameters on the dyeing performance is detailed in the supplementary data (Annex A.1). The results are given in Fig. 2. Analysis of the contour plots was used to estimate the optimal values for the studied response (K / S). Figure 2 shows the variation of the color yield as a function of two variables while keeping the third constant. The results suggest that high K / S values can be reached for high temperatures (80 ° C) and low pH (3) while the variation in the dyeing time did not significantly affect the response.

Response optimization and validation of the model

Optimization of the response was also performed using the Minitab software. The objective is to determine the optimal experimental conditions that give the maximum strength color (K/S) obtained by dyeing the polyamide with the *D. graveolens* aqueous extract. The results are summarized in Fig. 3 and indicates that the optimum level is reached for a dye bath at pH 3, for a temperature of 80 ° C and a dyeing duration time of 90 min. These optimal conditions lead theoretically to a colour strength (K/S) equal to 7.50. To validate this result, a polyamide dyeing test with the aqueous extract of *D. graveolens* under the optimum conditions given above was carried out in triplicate. An average value of K/S equal to 7.49 was found, which indicates that the experimental result corresponds to the theoretical optimum.

Hence, the model is validated. Fastness properties (washing, light and rubbing) of the polyamide fabric dyed with the aqueous extract of the *D. graveolens* are given in Table 4. The results show that the resistance to washing and rubbing are excellent. However, the light resistance is less good, which, according to the literature, is one of the main limits of natural dyeing (Faidi et al., 2016). However, post-treatments can be considered to improve the light resistance (Moussa et al., 2018).

Evaluation of biochemical activities

Anti-oxidant activity

- Assay of DPPH radical scavenging activity

The chemical characterization of the aqueous extract generated by the hydrodistillation of *D. graveolens* revealed the presence of polyphenols and flavonoids which are known for their antioxidant power; the latter has been evaluated for the aqueous extract and the result is given in Fig. 4.

Compared to the quercetin solution, which is the positive reference, the aqueous extract has a significant antioxidant power. Indeed, the inhibitory concentration IC_{50}, was evaluated at 0.022 $mg.mL^{-1}$ for the aqueous extract against 0.013 $mg.mL^{-1}$ for the standard solution.

- ORAC test

The principle of the ORAC test is to assess the "protective" effect of an antioxidant sample on the degradation process of fluorescein, initiated by the addition of AAPH as radical peroxyl generators ($ROO^{\bullet}$). The reaction is simple in concept but complex in practice. A competition between the reaction of targets and antioxidants with $ROO^{\bullet}$ forms the basis of the test: Fluorescein is an intensely fluorescent target in its native form. When attacked by peroxyl radicals, the fluorescence is lost. The antioxidants slow the loss of fluorescence by quenching peroxyl radicals via the transfer of hydrogen atoms or the addition of radicals (Prior et al, 2005). The reaction is followed by recording the fluorescence over time as shown in the figure (5a). The curve in the figure (5b) represents the measurement of the AUC_{net} at different concentrations of the aqueous extract. A strong reactivity of the aqueous extract is observed at concentrations between 0.01 and 0.05 mg. mL^{-1}, leading to AUC_{net} of 90 at 0.05 $mg.mL^{-1}$. This can be explained by the high content of polyphenols present in the aqueous extract and which are known for their antioxidant potential.

The evaluation of the anti-free radical and antioxidant power revealed important results at convergent concentrations and this could be correlated to the data obtained according to the test of the content of phenols / reducing agents.

Antibacterial activity

The main results of the antibacterial tests of the aqueous extract are gathered in Table 5.

These tests highlight the important antibacterial activity of the aqueous extract mostly against *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Staphylococcus epidermidis* with inhibition zone diameters of 21, 18 and 17 mm respectively at concentration of 10 mg. mL^{-1}.

Anti-inflammatory activity

The in-vivo anti-inflammatory activity of the aqueous residue was evaluated by xylene-induced ear edema in mice model. As the Fig. 6 shows, a weak inhibition of the control due to a developed increase in the average thickness of the ear following the topical application of xylene on the right ear of the control group. Whereas, the aqueous extract, 30 min before the application of xylene, significantly suppressed the edema of the ear compared to the control group with the highest percentage of inhibition at the 10 mg. kg^{-1} (75.59%). The aqueous extract also showed a high anti-inflammatory potential compared to

those of dexamethasone, the reference drug, the effect of which did not exceed 53.49%, contrary to aspegic reference which presents the highest anti-inflammatory potential (91.48%).

Previous research on the genus *Inula* has indicated that the characteristic compounds of this genus are sesquiterpenes lactones (Seca et al, 2014). The major part of experimental studies is focused on these products, in particular their cytotoxic and anti-inflammatory activities (Seca et al, 2014). In addition, experimental studies of *D. graveolens* from Turkey have focused on isolated lactone sequiterpenes such as ivalin, 8-epi-inuviscolide, 8-epi-xanthatin-1β, 5β-epoxide, which are involved in the cytotoxic and antibacterial effects (Topçu et al, 1993), in addition to flavonoids and terpenoids which revealed an antiproliferative effect of Jordan *D. graveolens* (Abu-Dahab and Affifi, 2007). Consequently, the significant biological effects (antibacterial, cytotoxic and anti-inflammatory effects) of the aqueous residue obtained from the hydrodistillation of *D. graveolens* could be attributed to the presence of these products.

Cytotoxic test

The cytotoxicity of the aqueous extract was carried out against healthy skin fibroblasts CCD-45 SK (ATCC® CRL 1506). After having studied the dye power and the biological potential of the aqueous residue of hydrodistillation, it is interesting to know its toxicity on healthy fibroblast cells of the skin. The results shown in Fig. 7 proved the non-cytotoxicity of the aqueous extract towards healthy skin fibroblasts in concentration range (≤ 0.2 mg. mL^{-1}) showing anti-free radical and antioxidant activities. This could be a factor which reinforces its added value.

Conclusion

The aqueous extract generated by the hydrodistillation of *D. graveolens*, the discharge of which presents a risk of environmental pollution, was used for the dyeing of the polyamide fiber and was evaluated for its biochemical potential. Thanks to its high content in polyphenolic compounds, the aqueous residue revealed a good tinctorial power. Among these polyphenols, two coloring molecules, catechin and quercetin, were identified and quantified. The optimum dyeing conditions were evaluated at pH of 3, temperature of 80 °C and duration time of 90 min for which the color yield (K/S) is equal to 7.5 and the fastness properties of rubbing, light and washing were estimated at 4, 3 and 4–5, respectively. Moreover, significant antioxidant, antibacterial and anti-inflammatory activities and non-cytotoxicity against healthy skin fibroblasts have been found for the aqueous residue.

Finally, the results obtained in this study are promising to revive the age-old art of dyeing with natural stuffs and reduce the risks of pollution that these liquid residues can cause. By the way, this encourage us to go ahead in the development of research on smart textiles taking advantage of the biological safety that this Tunisian *D. graveolens* aqueous residue revealed.

Abbreviations

HPLC
High Performance Liquid Chromatography
D. graveolens
Dittrichia graveolens
UV-vis
Ultraviolet-visible
IR
Infrared
RSM
Response Surface Methodology
CCD
Central Composite Design
ANOVA
Analysis of variance
DPPH
2,2-diphenylpicrylhydrazyl
ORAC
Oxygen Radical Absorbance Capacity
IC
Inhibitory Concentration
AAPH
2,2-azobis-(2-aminopropane)-dihydrochloride.
PBS
Phosphate Buffered Saline
AUC
Area under the curve
CFU
Colony Forming Unit
MTT
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO

Dimethyl Sulfoxide
$mg.EqGA.g^{-1}$ extract
milligram of equivalents of Gallic Acid per gram of extract
$mg.Eq.C.g^{-1}$ extract
milligram of equivalents of Catechin per gram of extract
T
Temperature
t
duration

Declarations

Ethics approval and consent to participate

The animals were treated in accordance with guidelines established by the European Union for the Use and Care of animals (CEC Council 86/609).

Consent for publication

Not applicable

Availibility of data and materials

Not applicable

Competing interests

The authors declare that they have no competing inerests.

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Authors' contributions

All authors read and approved the fnal manuscript.

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Tables

Due to technical limitations, the tables are provided in the Supplementary Files section.

Figures

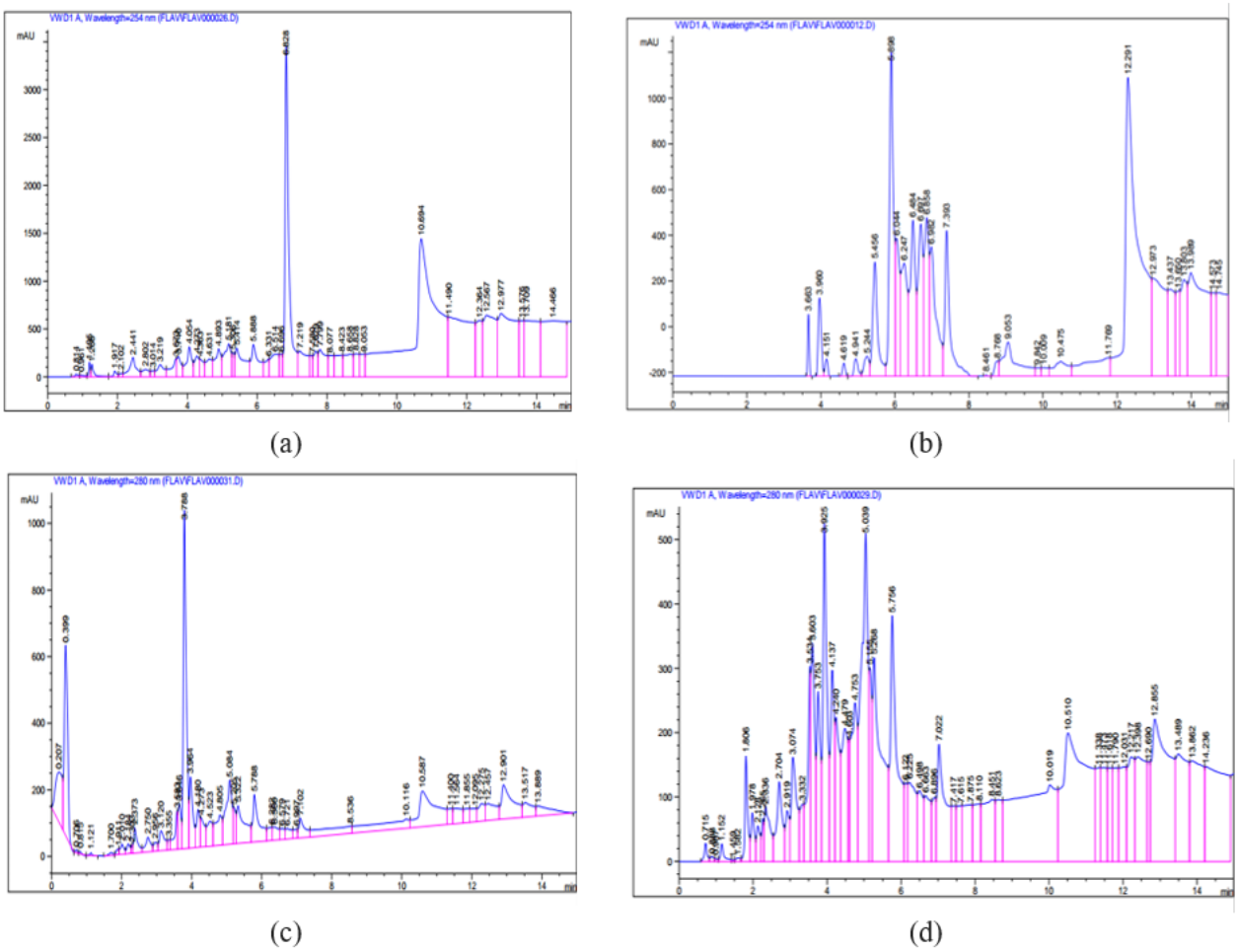

Figure 1

Aqueous residue chromatogramms for quercetin identification at 254 nm (before (a) and after (b) standard addition) and catechin identification at 280 nm (before (c) and after (d) standard addition)

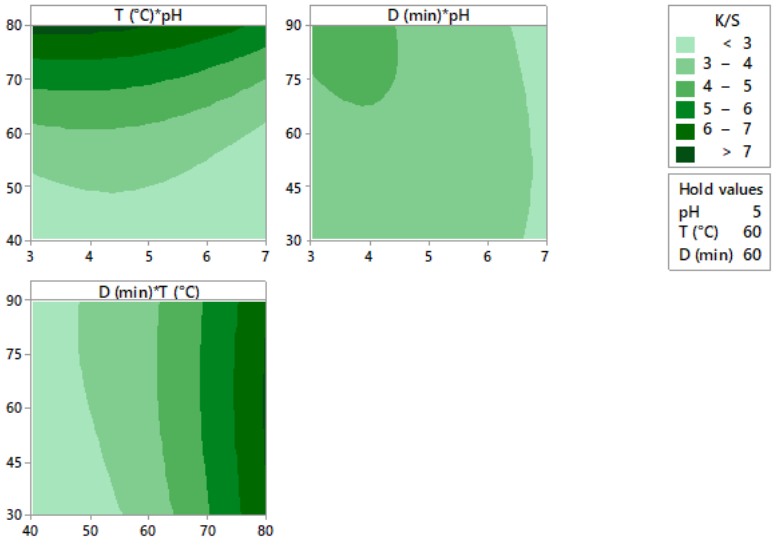

Figure 2

Contour plots of response

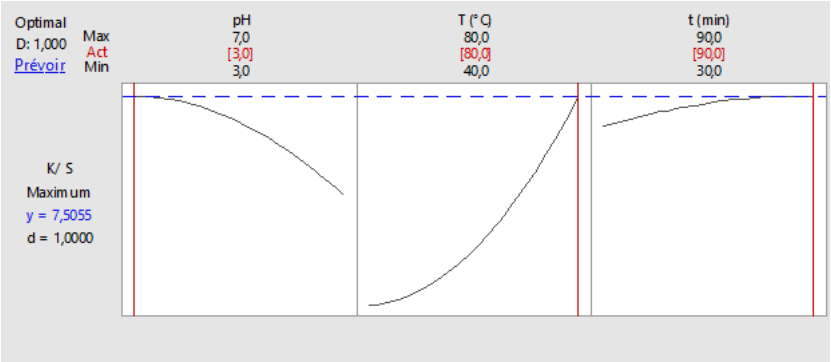

Figure 3

Response optimization for the colour yield parameter (K/S)

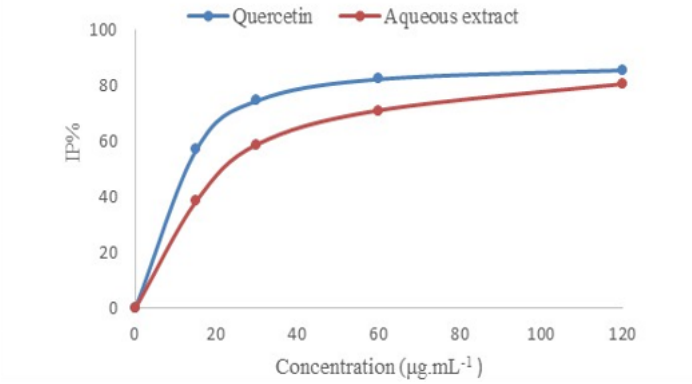

Figure 4

Antioxidant activity of the aqueous extract of D. graveolens

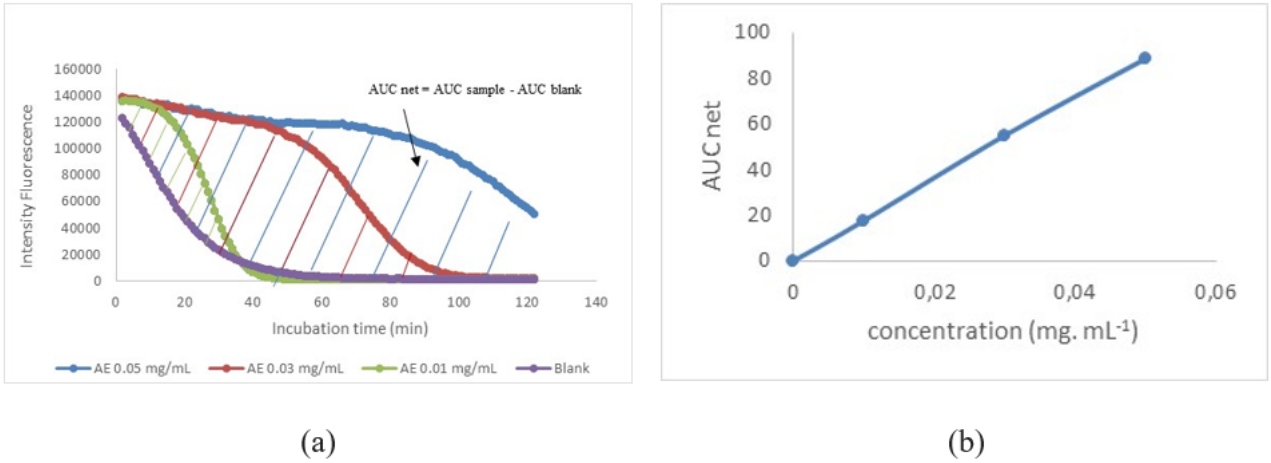

Figure 5

Antioxidant activity of the D. graveolens aqueous extract according to the ORAC test: (a) Profile of decrease in fluorescence intensity with different concentrations of D. graveolens aqueous extract (AE), (b) D. graveolens aqueous extract dose response curve.

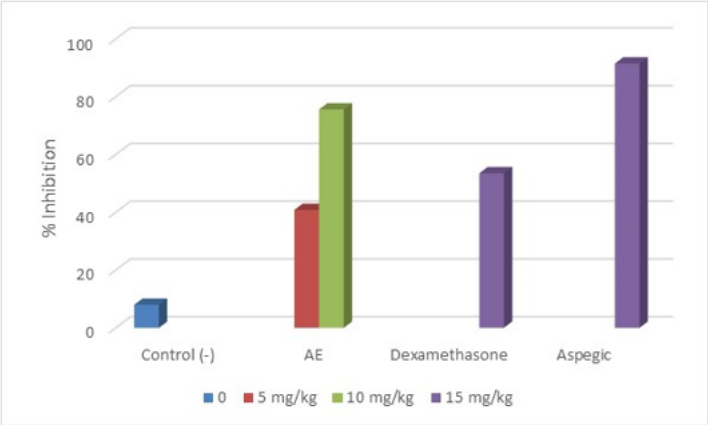

Figure 6

Anti-inflammatory effect of D. graveolens aqueous residue on xylene induced ear edema in mice in comparison to the control group and the two references group dexamethasone and Aspegic.

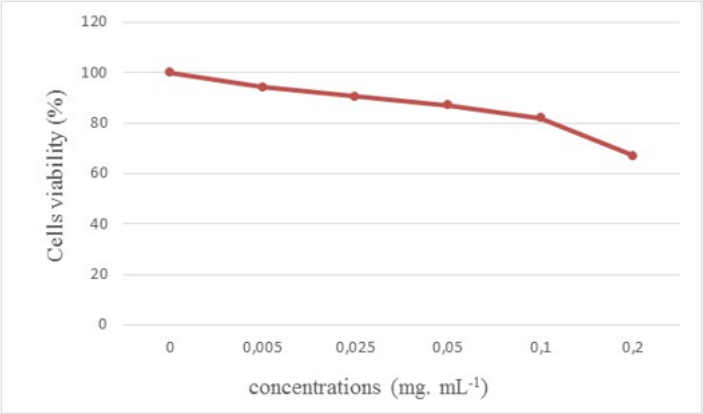

Figure 7

Viability of healthy skin-fibroblasts and HepG2 cells in the presence of increasing concentrations of the D. graveolens aqueous extract determined by MTT Assay

Supplementary Files

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