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Ammodaucus leucotrichus and *Citrullus colocynthis* from algerian Sahara: Ethnopharmacological application, phytochemical screening, polyphenols content and antioxidant activity of hydromethanolic extracts

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1. Introduction

The Sahara is the largest of the deserts but also the most expressive and typical by its extreme aridity, that is to say the one in which the desert conditions reach their greatest hardships. The vegetation mat is discontinuous and very irregular, and the plants use sites where water supply is somewhat less unfavorable than elsewhere (Le Houerou, 1990; Boukerker et al., 2016). The vegetation of the arid zones, particularly of the Sahara, is very sparse, generally naked and desolate, trees are scarce and scattered, and the grasses appear only during a very short period of the year, when conditions become favorable (Schiffers, 1971; Boukerker et al., 2016). The Saharan climate is characterized in particular by the weakness and irregularity of precipitation, intense brightness, strong potential evapotranspiration and high thermal amplitude. These characteristics create drastic conditions that cannot be tolerated by the living beings of these ecosystems, which result in phytochemical adaptation and the appearance of new protective molecules against the extreme Saharan climate (Sitouh, 1983; Chehma and Djebar, 2008).

In addition to their ecological and forage importance, spontaneous plants have many uses, traditionally practiced by the local population, in terms of pharmaceuticals, food and domestic use. These plants have the ability to synthesize many compounds called secondary metabolites and thus constitute an immense reservoir of compounds of great chemical diversity, possessing a wide range of biological activities (Jean and Jiri, 1983). Such is the case of plant

polyphenols which are widely used in therapeutics as well as antioxidants effects. In recent years, studies of antioxidant activities of medicinal plants have increased remarkably because of their potential to be used as sources of rich and natural antioxidants (Chaouche et al., 2015). In this context, our work is devoted to an ethnopharmacological investigation, phytochemical study and to the evaluation of the antioxidant activity of the hydro-methanol extracts of two Saharan plants from Bechar (Algeria). These include: *Ammodaucus leucotrichus* (Apiaceae) and *Citrullus colocynthis* (Cucurbitaceae).

2. Material and methods

2.1. Study site

The ethnopharmacological study was carried out from October 2015 to February 2016 in Bechar in the south-western region of Algeria. It is the wilaya of Bechar (31°37'00"N and 2°13'00"E). The climate of the Bechar region is of the continental desert type. Its main characteristic is the significant thermal differences between winter (2–3 °C) and summer 45 °C (Haddouchi et al., 2016). This locality was chosen for the geographical location of the studied plants and the fact that the traditional healers are organized and reputed to have a good knowledge on the use of the medicinal plants.

2.2. Ethnopharmacological investigation

The following plants were studied: *Ammodaucus leucotrichus* and *Citrullus colocynthis*. For that, the specimens were deposited in their herbarium. The investigation was carried out with 50 interviewed people, including herbalists and healers inhabitants of towns and villages of Bechar. All interviewees were informed about the purpose of this study. Information on the uses of the both plants was collected using a questionnaire through conversations with the respondents using the national language (Arabic). The collected data included the local common name, parts used,

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preparation and popular use. All information was collected and analyzed.

2.3. Plant material

The fruits of both plants studied were collected in March 2016 from Bechar. Plant samples were identified and then dried in the dark at room temperature in an aerated room for a period of 10–15 days.

2.4. Preparation of plant extracts

Extractions were carried out by maceration or decoction using a water/methanol mixture (20:80) (Haddouchi et al., 2016). Extractions continue 24 h for maceration and 2 h for decoction. After that, each solvent-extract mixture was evaporated by a rotary evaporator. This type of extraction was used to extract the polyphenols from the polar solvent, water/methanol mixture.

The dry yield of the plants was determined by calculating the following ratio:

$$\text{Yield}(\%) = [W_1 - W_2/W_3] \times 100$$

W_1 : weight of the flask after evaporation; W_2 : weight of the empty flask before evaporation; W_3 : weight of the starting vegetable matter.

2.5. Phytochemical screening

In order to demonstrate the presence or absence of certain compounds belonging to the chemical families of the secondary metabolites, we have carried out specific phytochemical tests based on staining, turbidity or precipitation reactions using the methods described in the literature. All tests are briefly described below:

2.5.1. Detection of flavonoids

Ten drops of concentrated hydrochloric acid and a few milligrams of magnesium turnings were added to 0.5 ml of each extract. The pink-red or yellow coloration, after three minutes of incubation at room temperature, indicates the presence of flavonoids (Haddouchi et al., 2016).

2.5.2. Detection of tannins

Eight drops of diluted ferric chloride solution (1%) were added to 1 ml of each extract. After a few minutes of incubation at room temperature, the ferric chloride develops a greenish coloring which indicates the presence of catechic tannins or blackish blue which reveals the existence of gallic tannins (Iqbal et al., 2015; Haddouchi et al., 2016).

2.5.3. Detection of anthocyanins

For this test, 5 ml of sulfuric acid (10%) and then 25% ammonium hydroxide were added to 1 ml of the diluted extract. The accentuated coloring by acidification and the turning to purplish-blue in a basic medium revealed the presence of the anthocyanins (Oloyede, 2005).

2.5.4. Detection of coumarines

A quantity of 5 mL of each extract was evaporated to dryness. The residue thus obtained was taken up in hot water. One volume of this aqueous phase was added with a solution of ammonia (10%) and another volume was kept as a control. The appearance of fluorescence after observation under UV at 366 nm indicated the presence of coumarins (Kumar et al., 2013).

2.5.5. Detection of alkaloids

Two milliliters of hydrochloric acid (1%) were added to 1 ml of each extract, the whole is heated on a water bath and then, each extract was divided into two equal volumes. One volume was processed by Mayer's reagent, the other by Wagner's reagent. The positive results were revealed by the formation of a yellowish-white precipitate by the Mayer's reagent or orange-red to brown for the Wagner's reagent (Iqbal et al., 2015).

2.5.6. Detection of saponins

A volume of 2 ml of each extract were placed in test tubes which were adjusted to 5 ml with distilled water. The tubes were shaken for 15 s in the lengthwise direction and leaved to stand for 20 min. Results were positive when the height of the foam is greater than 1 cm (Haddouchi et al., 2016).

2.5.7. Detection of terpenoids

A quantity 0.5 ml of chloroform and 0.7 ml of concentrated sulfuric acid were added to 1 ml of each extract. The green–blue color revealed the presence of steroidal heterosides as well as the green–violet color reveals the presence of the terpene heterosides (Haddouchi et al., 2016).

2.5.8. Detection of reducing compounds

A volume of 2 ml of Fehling liquor were added to 2 ml of extract and then, the whole was incubated 8 min in a boiling water bath. The appearance of a red brick precipitate indicated the presence of the reducing compounds (Sabri et al., 2012).

2.6. Total polyphenols content

The polyphenols were assayed according to the method described by Dewanto et al. (2002). The principle of the method is based on the oxidation of phenolic compounds by the Folin–Ciocalteu reagent, which is a complex mixture of phosphotungstic acid and yellow phosphomolybdic acid. This oxidation leads to the formation of a new blue molybdenum–tungsten complex which absorbs at 750 nm. Briefly, 100 μ l of each extract were mixed with 2 ml of a freshly prepared sodium carbonate solution (2%), the whole was agitated by a vortex during five minutes. After that, 100 μ l of the Folin–Ciocalteu reagent (1N) were added to the mixture, the whole was left for 30 min at room temperature. The revelation was performed comparatively to a blank using a spectrophotometer at 750 nm. A standard range based on gallic acid was also prepared at concentrations ranging from 0 to 400 μ g/ml. The total polyphenol contents of the extracts were then expressed in milligrams gallic acid equivalent per gram of the dried sample (mg EAG/g DS).

2.7. Antioxidant activity

2.7.1. DPPH radical scavenging activity

The DPPH method is simple and fast. DPPH (2,2-diphenyl-1-picrylhydrazyl) is the most widely used substrate for the rapid and direct evaluation of antioxidant activity because of its stability in free radical form and the simplicity of the analysis. The DPPH method has several advantages in that it is independent, simple and fast. The test consists in putting the DPPH radical (of violet color) in the presence of the so-called molecules “Antioxidants” to measure their ability to reduce this radical. The reduced form (yellow) no longer absorbs, which results in a decrease in absorbance at this wavelength. We used the protocol of Prieto et al. (1999). So, for different concentrations of each extract, 50 μ l were added to 1950 μ l of a methanolic solution of DPPH at 6.34×10^{-5} M. For each concentration, a blank was prepared. In parallel, a negative control was also prepared by mixing 50 μ l of methanol with 1950 μ l of a methanolic solution of DPPH at the

same concentration used. After incubation in the dark for 30 min and at room temperature, the reduction of DPPH was accompanied by the passage of the violet color to the yellow color of the solution. Absorbance reading was performed at 515 nm using a spectrophotometer. The positive control used is ascorbic acid.

The results were expressed as percent inhibition (PI) which reflects the reduction in the color intensity of the mixture according to the formula:

$$PI = (A_{\text{control}} - A_{\text{extract}} / A_{\text{control}}) \times 100$$

PI: percent inhibition; A_{control} : negative control absorbance; A_{extract} : absorbance of the extract. The study of the variation of the antiradical activity as a function of the concentration of the extracts makes it possible to determine the concentration which corresponds to 50% inhibition (IC_{50}). A low IC_{50} value corresponds to a high efficiency of the extract.

2.7.2. Reducing power assays

The reducing activity of an extract was evaluated by the redox reaction between the extract and the transition metal ions, in particular iron. Potassium ferricyanide provides ferric ions (Fe^{3+}) which will be reduced to ferrous ions (Fe^{2+}) by the antioxidants present in the plant extract. The activity was determined according to the method described by Oyaizu (1986).

This method consists of mixing 1 ml of each extract at different concentrations with 2.5 ml of 0.2 M phosphate buffer at pH 6.6 and 2.5 ml of a 1% potassium ferricyanide solution. The mixture obtained was incubated for 20 min at 50 °C and then, 2.5 ml of 10% trichloroacetic acid were added to make end the reaction. The mixture was centrifuged at 650 g for ten minutes at room temperature. After that, 2.5 ml of the supernatant were added to 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) iron chloride. The absorbance was reading at 700 nm against a blank. The results allowed us to calculate the effective concentration (EC_{50}), concentration of the corresponding extract with an absorbance equal to 0.5, obtained by the interpretation of the linear regression curve (optic densities according to the different concentrations). The activity of the extract was finally compared with that of the positive control, the butylhydroxyanisol (BHA).

2.8. Statistical analysis

All tests were conducted in triplicate and results were expressed as mean values \pm standard deviation. All analyses were performed in Microsoft Excel Program for Windows, 2007.

3. Results

3.1. Ethnopharmacological investigation

The results are presented in the Table 1. The percentage of respondents reporting knowledge of these plants and their medicinal uses was 100%. The study has generally identified about two diseases treated with *A. leucotrichus*, they had the hypertension and digestive disorders. More than 15 diseases can be treated with *C. colocynthis*: otitis, eczema, intestinal parasitism, constipation, inflammation, diabetes, jaundice, fever, ascites, bites, epilepsy, hair loss, rheumatism, neoplasm, gout, arthritis, hemorrhoids and varicose veins. For traditional preparations, local communities used fruits *A. leucotrichus* as decoction or infusion with a rate of 44.44%; that extracts were administrated orally. However, for *C. colocynthis*, the fruits and their seeds were most used (83.32%) by dermal or by oral route. All interviewees insisted that the plant harvest season for medicinal use is spring/summer for *A. leucotrichus* and spring/autumn for with *C. colocynthis*.

Table 1
Results of the ethnopharmacological investigation of *Ammodaucus leucotrichus* and *Citrullus colocynthis*.

| Botanical name | Local name | English name | Family name | Parts used | Preparation | Popular use | Administration | Season of harvest |
|--------------------------------|---|--------------|---------------|---|-----------------------|--|------------------------|-------------------|
| <i>Ammodaucus leucotrichus</i> | Kammun es-sofi, el massoufa, Oum draiga, (Arab) | Sahara cumin | Apiaceae | Fruits (44.44%) Leaves (38.88%) Aerial part (16, 68%) | Decoction or infusion | Hypertension (88.33%), Digestive tract and stomach ailments (38.88%). | Oral use | Spring/Summer |
| | Akaman (Targui) | Bitter apple | Cucurbitaceae | Fruits (13.33%) | Infusion or decoction | Anti-otitis, anti-eczema, against intestinal parasites, constipation against urogenital disorders | Dermal use | Spring/autumn |
| <i>Citrullus colocynthis</i> | Handal /Hadag (Arab) | | | Fruits (19.99%) | Raw | Antiparasitic-insecticide (tapeworm, scorpion), anti-inflammatory | Dermal use | |
| | | | | Seeds (10%) | Powder | Anti-diabetic, antiparasitic-insecticide, leukemia, jaundice, fever, ascites, biliary disorders | Oral use | |
| | | | | Seeds (40%) | Oil | Treatment of bites (snake, scorpion), epilepsy, to promote the growth of hair and to blacken gray hair | Dermal use | |
| | | | | Pulps (13.33%) Roots (3.33%) | Powder Dough | Hemorrhoids and Varicose Veins The treatment of rheumatism, antineoplastic, anti dropsy, anti-gout, anti-arthritis and can be a cure for cerebral congestion, rheumatism and sciatica | Oral use Dermal use | |

3.2. Yields

Hydromethanolic extracts (water/methanol, 20/80) were prepared by maceration (WMM) or by decoction (WMD) of the fruits of *A. leucotrichus* and *C. colocynthis*. The various yields of hydromethanolic extracts obtained are reported in Table 2. For the extraction, methanol was mixed with distilled water in the ratio of 80/20 (v/v). This mixture is the most effective and widely used in the literature (Djeridane et al., 2007; Harborne, 1998; Li et al., 2008; Wong et al., 2006). For *A. leucotrichus*, the hydromethanolic extracts prepared by maceration and decoction showed yields of the order of $16.84 \pm 0.49\%$ and $11.04 \pm 0.29\%$, respectively. On the other hand, we noted yields in the order of $10.75 \pm 0.25\%$ and $22.86 \pm 0.06\%$ for the hydro-methanolic extracts of *C. colocynthis* which were prepared by maceration and decoction, respectively.

3.3. Phytochemical screening

Phytochemical tests were carried out on hydromethanolic extracts prepared from the fruits of *A. leucotrichus* and *C. colocynthis* using maceration or decoction methods. The detection of these chemical compounds was based on tests of component solubility, precipitation, flocculation and turbidity reactions, by a specific color change or an examination under ultraviolet light. The experimental results of the phytochemical tests carried out on the crushed plant material are shown in the Table 3. We note that the water/methanol mixture leads to the same compounds using maceration or decoction methods for the both studied plant. For *A. leucotrichus*, presence of terpenes can be explained by the presence of terpene derivatives of polar nature such as mono terpenes. In addition, *A. leucotrichus* was very rich in alkaloids with a medium presence of polyphenols, but it was very poor in saponins. For *C. colocynthis*, the phytochemical tests revealed the presence of those metabolites except saponins.

3.4. Total polyphenols content

The total phenol content was determined from the equation of the linear regression of the gallic acid calibration curve: $y = 0.005x + 0.185$ ($R^2 = 0.996$) and the results were expressed in mg gallic acid equivalents per g of dried sample (mg GAE/g DS). Table 2 summarizes the results for both species. From the obtained results, we observed a high content of polyphenols in the hydro-methanolic extract of the fruits of *C. colocynthis*. The extract prepared by maceration contained 113.5 ± 0.3 mg GAE/g DS; however, the extract obtained by decoction contained 127.6 ± 0.77 mg GAE/g DS. For *A. leucotrichus*, the fruits macerated by hydromethanolic solvent revealed polyphenols content of 10.32 ± 0.17 mg GAE/g DS. While, the polyphenols content of the same extract obtained by decoction, was 16.65 ± 0.22 mg GAE/g DS.

3.5. Antioxidant activity

The evaluation of the antioxidant activity of the hydromethanolic extracts of the fruits of *A. leucotrichus* and *C. colocynthis* was carried out by two conventional methods in order to test these

Table 3

Results of phytochemical tests of hydromethanolic extracts of *Ammodaucus leucotrichus* and *Citrullus colocynthis*.

| | <i>A. leucotrichus</i> | | <i>C. colocynthis</i> | |
|--------------------|------------------------|-----|-----------------------|-----|
| | WMM | WMD | WMM | WMD |
| Flavonoids | + | + | ++ | ++ |
| Tannins | ++ | ++ | ++ | ++ |
| Coumarines | ++ | ++ | + | ++ |
| Alkaloids | +++ | +++ | ++ | ++ |
| Saponins | – | – | – | – |
| Terpenoids | + | + | – | – |
| Reducing compounds | ++ | ++ | ++ | ++ |

–: absence; +: presence in small quantity; ++: presence in average quantity, +++: Presence in large quantities, WMM: water/methanol extract by maceration, WMD: water/methanol extract by decoction.

extracts with respect to the various reaction mechanisms involved in these antioxidant tests.

3.5.1. DPPH radical scavenging activity

The DPPH radical is one of the most widely used substrates for the rapid and direct evaluation of antioxidant activity due to its stability in radical form and the simplicity of this analysis. Fig. 1 shows the antioxidant power of the hydromethanolic extracts of the fruits of *A. leucotrichus* and *C. colocynthis* compared to that of the positive control used (Ascorbic acid) with respect to the scavenging of the free radical DPPH.

The antioxidant power of the extracts of the two plants studied and ascorbic acid was proportionally increased regarding the variation of concentration. According to our results, we observed that the hydromethanolic extracts of the fruits of both plants showed an antioxidant activity by scavenging of the free radical DPPH. The traced graphs translated an exponential appearance which ends by a stationary phase; this is explained by the reducing of DPPH radical to its non-radical form. By comparison of the antioxidant power, the fruit extracts of *A. leucotrichus* and *C. colocynthis* were less active than ascorbic acid (positive control).

The values of the concentrations corresponding to the inhibition of 50% of the free radical DPPH (IC_{50}) are summarized in Table 4. The extracts obtained by maceration and decoction of *A. leucotrichus* fruits had important IC_{50} values of 5.702 ± 0.373 and 3.089 ± 0.076 mg/ml, respectively. We observed that the hydro-methanolic extract by decoction of *A. leucotrichus* showed the most remarkable antioxidant effect compared to that obtained by maceration. The extracts of *C. colocynthis* showed scavenging of the free radical DPPH at 50% by the both following concentrations of 3.168 ± 0.633 and 3.335 ± 0.435 obtained by maceration and decoction, respectively.

3.5.2. Reducing power assays

The values of the optical densities obtained allowed drawing curves for each hydromethanolic extract obtained by maceration and decoction. In this test, the increase in absorbance reflects an increase in the reducing power of the extracts tested. The results shown in Fig. 2 have shown that the reduction capacity was proportional to the increase in the concentrations used.

Table 2

Yields of dried extract and total polyphenols content of hydromethanolic extracts of *Ammodaucus leucotrichus* and *Citrullus colocynthis*.

| | Yields (%) | | Total polyphenols content mg GAE/g DS* | |
|--------------------------------|------------------|------------------|--|------------------|
| | WMM | WMD | WMM | WMD |
| <i>Ammodaucus leucotrichus</i> | 16.84 ± 0.49 | 11.04 ± 0.29 | 10.32 ± 0.17 | 16.65 ± 0.22 |
| <i>Citrullus colocynthis</i> | 10.75 ± 0.25 | 22.86 ± 0.06 | 113.5 ± 0.3 | 127.6 ± 0.77 |

WMM: water/methanol extract by maceration, WMD: water/methanol extract by decoction.

*mg gallic acid equivalents per g dried sample.

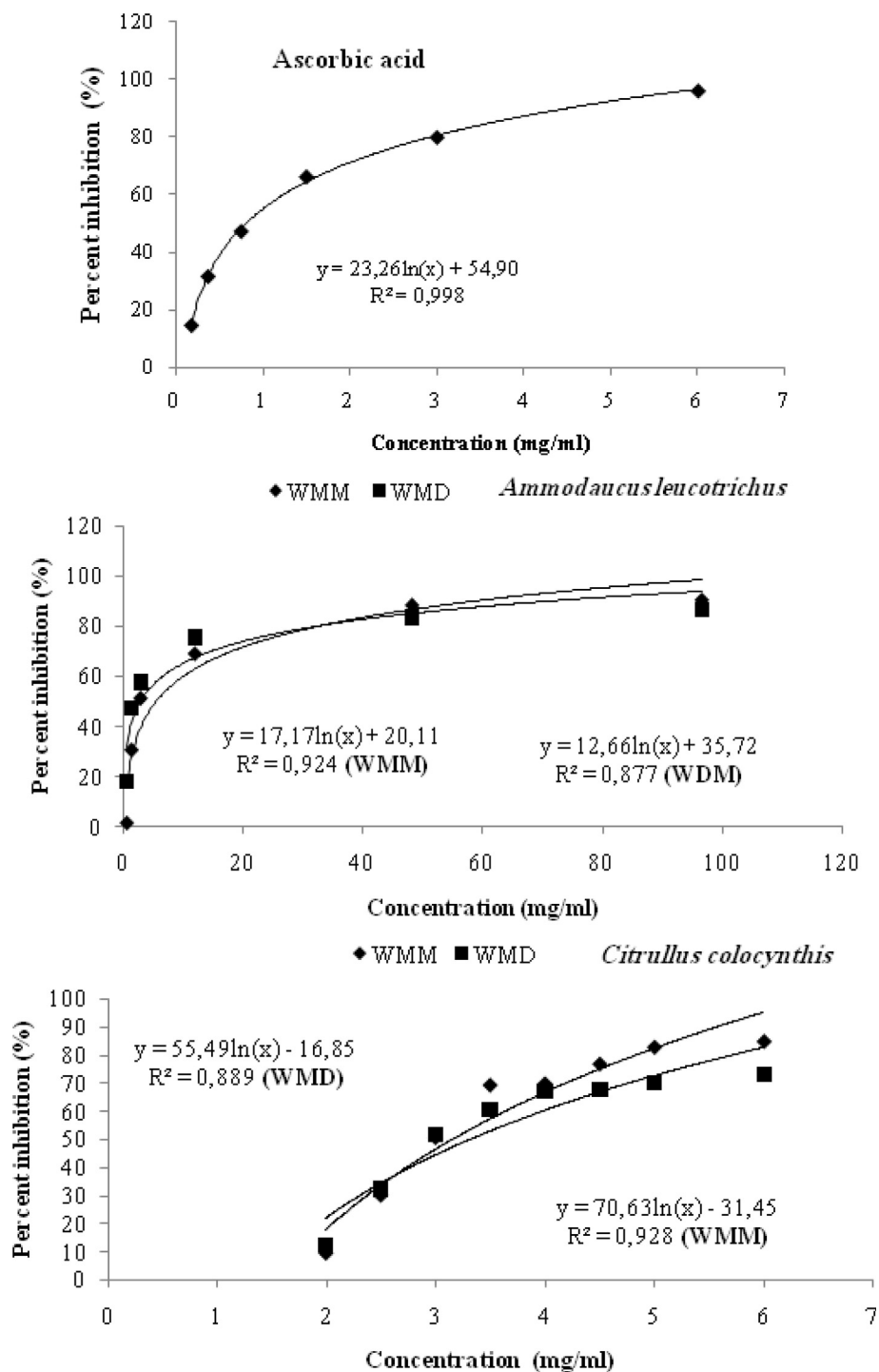


Fig. 1. Percentage of inhibition of the free radical DPPH as a function of the different concentrations of ascorbic acid and hydromethanolic extracts of *Ammodaucus leucotrichus* and *Citrullus colocynthis* (decoction and maceration).

Table 4

IC₅₀ values for reduction of radical DPPH and EC₅₀ of reducing power of hydromethanolic extracts of *Ammodaucus leucotrichus* and *Citrullus colocynthis* (mg/ml).

| | IC ₅₀ DPPH | | EC ₅₀ reducing power | |
|--------------------------------|-----------------------|---------------|---------------------------------|--------------|
| Ascorbic acid | 0.81 ± 0.04 | | / | |
| BHA | / | | 0.42 ± 0.12 | |
| | WMM | WMD | WMM | WMD |
| <i>Ammodaucus leucotrichus</i> | 5.702 ± 0.373 | 3.089 ± 0.076 | 25.93 ± 1.82 | 72.28 ± 0.88 |
| <i>Citrullus colocynthis</i> | 3.168 ± 0.633 | 3.335 ± 0.435 | 30.68 ± 1.56 | 23.85 ± 2.77 |

WMM: water/methanol extract by maceration, WMD: water/methanol extract by decoction.

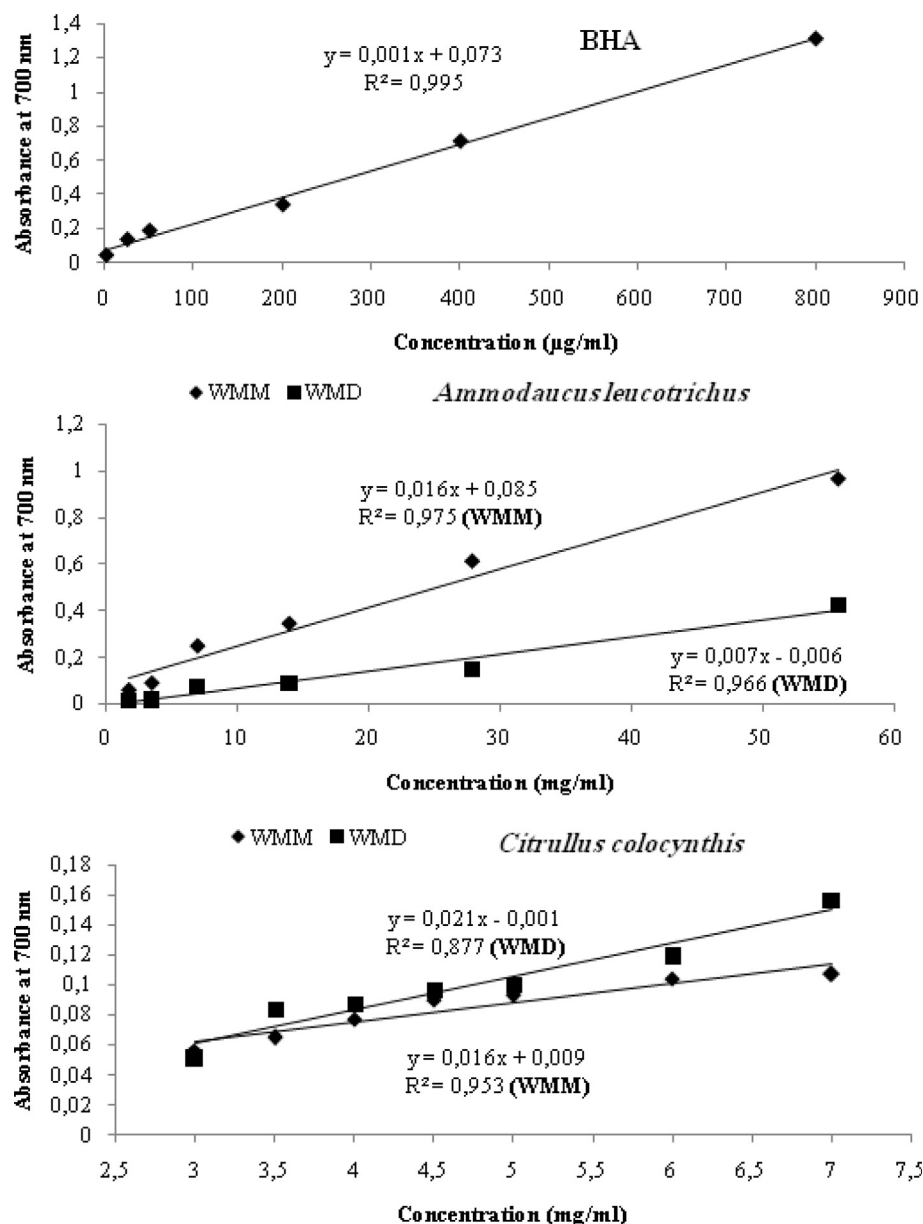


Fig. 2. Reducing power as a function of the different concentrations of BHA and hydromethanolic extracts of *Ammodaucus leucotrichus* and *Citrullus colocynthis* (decoction and maceration).

In order to compare the antioxidant activity of the extracts tested by this method, we calculated the EC_{50} concentration. The results obtained were illustrated in Table 4, from which we noted that the hydromethanolic extracts of the fruits of *A. leucotrichus* showed a low reducing power comparatively with the positive control used (BHA) at different concentrations, 25.93 ± 1.82 mg/ml (WMM) and 72.28 ± 0.88 mg/ml (WMD). The ability to reduce iron was lower compared to the positive control for hydromethanolic extracts of *C. colocynthis*. It resulted in an EC_{50} of 30.68 ± 1.56 mg/ml and 23.85 ± 2.77 mg/ml for WMM and WMD, respectively.

4. Discussion

The two studied plants, *Ammodaucus leucotrichus* and *Citrullus colocynthis*, were collected in Saharan area (Bechar in south-western region of Algeria). With an area of 2 million km^2 , Saharan ecosystems account for 87% of the surface area of Algeria. The

Saharan space is made up of several distinct geomorphological units. This space is distinguished by extreme climatic conditions (high temperatures, very low rainfall, even non-existent and a very marked aridity). In terms of vegetation, the Saharan ecosystem contains 2,800 taxa with a high rate of endemism (MATE-PNUD-FEM, 2015). For this reason, we chose the studied plants and the study site of the ethnopharmacological survey.

Several ethnopharmacological investigations have noted the traditional use of *A. leucotrichus* to treat gastric diseases and digestive tract disorders in Algeria (Didi et al., 2003) and also in Morocco (Merzouki et al., 2000; Fakchich and Elachouri, 2014). Interrogates told us that *A. leucotrichus* is also used for the treatment of arterial hypertension, putting the fruit under the tongue during a peak of tension felt by the patient. In fact, Takagi et al. (2005) studied the vasodilating effect of perillaldehyde on rats, this plant compound exerts a vasodilator effect by blocking the Ca^{2+} channels of aortes isolated from experimental rats. On the other hand, our results have been reported by some studies on *C. colocynthis*,

including the treatment of diabetes, cathartic, hepatic and biliary diseases (Panda, 2000; Al-Qura'n, 2009). In addition, Al-Snafi (2016) collected most of the studies done on the effects of this plant among them which confirmed their traditional uses.

About the yield *A. leucotrichus* fruits, Sifi et al. (2015) reported a maceration yield of 20.4% of *A. leucotrichus* fruits using methanol. This high level may be due to the solvent used. Furthermore, the yield obtained for hydromethanolic extracts from the fruits of *C. colocynthis* was not the same of this obtained by Benariba et al. (2013) which reported a yield of 4.2%.

Extractions were carried out by maceration or decoction using a water/methanol mixture (20:80). Since this solvent has a polar nature, the metabolites that have been revealed were of a polar nature (flavonoids, tannins, coumarins, alkaloids and reducing compounds). In this context, Najafi et al. (2010) revealed that the ethanolic extract of *C. colocynthis* fruits contained tannins, alkaloids, flavonoids, reducing sugars and additionally the presence of saponins. According to Benariba et al. (2013), catechic tannins and flavonoids were abundant in hydromethanolic extract, but terpenoids were present with low concentration. This minor difference in our results with previous ones may be related to the difference in harvesting station, climate of the area and the harvest season. The characteristics of the Saharan climate are essentially the result of the latitudinal situation in the tropics, which leads to high temperatures, and to the wind regime which results in hot, dry currents Ozenda, 2004).

In opposition to our results, Rached et al. (2010) showed that the polyphenol contents in *A. leucotrichus* fruits was 46.77 mg GAE/g DS. This is not in agreement with our result which may be due to the different solvents used for the extraction of polyphenols or environmental factors of the harvest station (Rat et al., 2016). Effectively; the extraction of phenolic compounds is governed by several factors which directly influence the levels of these molecules.

Most studies on the antioxidant activity of the fruits of *A. leucotrichus* were limited on essential oils. Conversely to our results, Rached et al. (2010) revealed that the aqueous extract of the aerial part of *A. leucotrichus* showed an IC_{50} of 0.045 ± 0.001 mg/ml, this dissimilarity may be due to the difference of the parts or the solvent used.

For *C. colocynthis*, at 2.5 mg/ml, Kumar et al. (2008) recorded an inhibition percentage of the free radical DPPH of $88.0 \pm 2.7\%$. However, at the same concentration we recorded an inhibition percentage of 30.16% (WMM) and 32.05% (WMM). Marzouk et al. (2010) found an IC_{50} between 0.020 mg/ml and 0.021 mg/ml of aqueous seed extracts with different maturation states. On the other hand, Jayaraman and Christina (2013), found that the maximum percentage inhibition of DPPH radicals by the methanolic extract was about 62% at 0.8 mg/ml, none the less we noted inhibition of 3.75% (WMM) and 4.14% (WMD) with the same concentration. Remarkably, the published paper by Benariba et al. (2013) on the same plant in Algeria but from a different geographic site, recorded an inhibition percentage of 74.5% at 2 mg/ml by an IC_{50} of 0.58 mg/ml. At the same concentration, we recorded a percentage inhibition of 9.67% (WMM) and 12.12% (WMD). Jayaraman and Christina (2013) revealed that the maximum antioxidant activity was found at the dose of 0.8 mg/ml. Our results are not in agreement with those obtained by previous works. By comparison, the harvest season and geographic station can influence the antioxidant properties of the extracts of the *C. colocynthis*. Kumar et al. (2008) worked on a plant from India harvested in October, Benariba et al. (2013) studied a plant which was harvested in September and November from Mechria (Algeria); this region is a very different than the Bechar region (our geographical station) in term of altitude and climate.

5. Conclusion

According to the ethnopharmacological investigation, hypertension and digestive disorders have been treated with *A. leucotrichus* in traditional medicine. More than 15 diseases can be treated with *C. colocynthis*, in particular, diabetes, and cathartic, hepatic and biliary diseases. The fruits of *A. leucotrichus* and *C. colocynthis* were very rich in polyphenols including flavonoids, tannins, coumarins. The content of polyphenols in the hydro-methanolic extract from the fruits of *C. colocynthis* was between 113.5 ± 0.3 and 127.6 ± 0.77 mg GAE/g DS. The antioxidant test showed that the concentrations between 3,089 and 5,702 mg/ml of the hydromethanolic extracts of the two plants can scavenge 50% of the free radical of DPPH. However, this is not the case with the method of reducing power. In summary, *A. leucotrichus* and *C. colocynthis* were very useful plants in traditional medicine. The hydromethanolic extracts, prepared from the fruits of *A. leucotrichus* and *C. colocynthis*, were rich in polar secondary metabolites with less antioxidant activity comparing with positive control.

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