

Impact of extraction method on alkaloid content and antioxidant activity of *Matricaria pubescens*

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ABSTRACT

Matricaria pubescens is a medicinal plant much appreciated by the people of the Saharan regions and widely used to treat many diseases. The present study was devoted to evaluate the effect of extraction technique, pH and nature of solvent on alkaloid content and antioxidant activity of *M. pubescens*. Alkaloid extraction was carried out by applying two techniques, maceration extraction and Soxhlet extraction, using several solvents at different pH (alkaline chloroform, acid methanol, neutral methanol and neutral ethanol). In the present study, the antioxidant activity of the alkaloid extracts of *M. pubescens* was evaluated using ferric reducing power, molybdate reducing power, DPPH radical scavenging activity, ABTS radical scavenging activity, β -carotene bleaching assay, hydroxyl radical scavenging activity (assessed by deoxyribose assay and HOSC assay) and oxygen radical absorbance capacity (ORAC). The obtained results show that the extraction rates obtained and the antioxidant activities of alkaloid extracts were affected by the extraction technique, pH and the solvent used. The highest extraction rates were obtained with the ethanolic neutral (Soxhlet) and methanolic acidified (maceration) extracts. The alkaloid extracts of *M. pubescens* exerted a reducing activity of ferric and molybdate ions. They also showed significant radical scavenger activity against DPPH, ABTS, hydroxyl, and peroxy radicals. This significant wealth of alkaloids and these various antioxidant activities could justify the traditional use of *M. pubescens*.

Introduction

Free radicals are often atoms or molecules or molecular fragments containing one or more unpaired electrons in their atomic or molecular orbitals, which give them significant reactivity. Indeed, a radical will always tend to fill its orbital by capturing an electron from other compounds to become stable. Thus, the new molecule formed becomes itself a free radical by losing its electron, triggering a cascade of chain

reactions which finally damages the living cell (Mukherji et al., 1986).

The main free radicals involved in human pathophysiological processes are the superoxide radical, the hydroxyl radical and the peroxy radical. Singlet oxygen and hydrogen peroxide are reactive oxygen species that are not free radicals but very reactive. Besides reactive oxygenated species (ROS), there are reactive nitrogenous species (RNS) whose major representative is nitric oxide (Huet and Duranteau, 2008; Bargagli et al., 2009). In low doses, ROSs are very useful for the organism and play

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important roles in various physiological mechanisms such as signal transduction, cell differentiation, apoptosis, immune defense against pathogens, destruction by apoptosis of tumor cells, regulation of capillary dilation, the functioning of some neurons, especially those of memory, fertilization of the ovum, regulation of genes and participate in the functioning of some enzymes (Koechlin-Ramonatxo, 2006; Roberts and Sindhu, 2009). Nevertheless, the excessive production of free radicals causes direct damage to biological molecules (lipids, proteins and DNA), but also secondary damages due to the cytotoxic and mutagenic character of the metabolites released in particular during the oxidation of lipids (Deby-Dupont et al., 2002). This damage results in oxidative stress which causes various diseases like Alzheimer's disease, Parkinson's disease, heart disease, diabetes mellitus, cancer (Halliwell and Gutteridge, 1990; Qazi and Molvi, 2018). In order to reduce the risk of chronic diseases and prevent their progression, it is necessary to enhance the body's natural antioxidant defenses or supplemented with food antioxidants. In this context, the search for new antioxidant compounds from natural sources has been increased. The alkaloids consist of the most abundant naturally occurring group of secondary metabolites in plants (Rehman and Khan, 2017). Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms and often have pharmacological effects. Many studies have demonstrated that alkaloids have biological activities, such as antioxidant, anti-microbial, anti-inflammatory, anti-cancer, and anti-virus (Yan et al., 2008). Moreover, the vast biological activities make many alkaloids prominent start line for chemical medicinal chemistry (Rehman and Khan, 2017).

Matricaria pubescens is a medicinal plant much appreciated by the inhabitants of the Saharan regions and used as a remedy against rheumatism, aches, coughs, allergies, eye diseases, dysmenorrhea, and gastro-intestinal disorders. In children, it is used against measles, ailments related to teething, fever and dermatosis (Maiza et al., 1995).

Various secondary metabolites like alkaloids, saponins, terpenoids, phytosterols (Djellouli et al., 2013), flavonoids and tannins (Metrouh-Amir et al., 2015) have been isolated from *M. pubescens*. Alkaloid compounds exert a wide range of biological effects. The study carried out by Metrouh-Amir and Amir, (2018) found that the alkaloids of *M. pubescens* exerted antinociceptive and anti-inflammatory activities.

The aim of this study was to determine the effect of extraction method and solvent on alkaloid contents and antioxidant activities of *M. pubescens*.

Materials and methods

Plant material

The entire plant was harvested during flowering in March 2016 at Ouargla area (Algerian Septentrional Sahara). Authentication and identification were made by the botanist J.P. Lebrun (National Museum of Natural History, Paris). A specimen of the plant was deposited at Laboratoire de Botanique d'Alger, Algeria (voucher number 644).

Plant processing and extraction of total alkaloids

The entire plant was air dried and ground and fractions with diameters of less than 250 µm were used for the extraction.

The extraction of alkaloids from *M. pubescens* was carried out using several protocols at different pH with various solvents (extraction in an alkaline medium, in an acid medium and in a neutral medium), by maceration and Soxhlet (Bruneton, 2009).

Extraction in an alkaline medium by maceration

The principle of this extraction protocol is based on the method of Bruneton, (2009). Ten grams of the powder were mixed with 100 mL of alkalinized chloroform at pH 9 with concentrated ammonium hydroxide. After 24 hours of maceration, the mixture was filtered. In order to extract the maximum amount of alkaloids, this phase was repeated three times. The recovered filtrate was partially concentrated then extracted with 100 mL of water acidified at pH 3 with HCl. This acid solution was adjusted to pH 9 with concentrated ammonium hydroxide, and extracted with chloroform. The organic phase recovered was dried with anhydrous sodium sulfate and then concentrated to dryness.

Extraction in an acid medium by maceration

The principle of extraction of alkaloids in an acid medium was inspired by the method of Yubin et al. (2014). An amount of 10 g of powder was mixed with 300 mL of methanol acidified to pH 4 with sulfuric acid. After 72 hours of maceration, the extract was filtered. In this case, the extraction was followed by vacuum distillation which eliminates the alcohol and leaves an acidic aqueous solution of alkaloid salts. The acidic aqueous solution recovered was made alkaline with concentrated ammonium hydroxide up to pH 9, and then extracted with chloroform until complete exhaustion. The extract obtained was dried with anhydrous sodium sulphate and evaporated to dryness.

Extraction in a neutral medium

The extraction of alkaloids in a neutral medium was carried out using two solvents, ethanol and methanol, by the Soxhlet apparatus and by maceration.

The extraction protocol with ethanol and methanol by Soxhlet was carried out according to Suau et al. (2002) method, with some modifications. Ten grams of the powder were mixed with 300 mL of alcohol (methanol or ethanol). After 8 h of extraction by Soxhlet apparatus, the mixture was filtered and evaporated. The residue was acidified with hydrochloric acid to pH 3. The acid extract was adjusted with concentrated ammonium hydroxide to pH 9, and then extracted with chloroform until complete exhaustion. The organic phase was dried with anhydrous sodium sulphate and evaporated to afford a crude extract of alkaloids.

The extraction protocol with ethanol by maceration is the next: The powdered sample (10 g) was extracted with 300 mL of ethanol by maceration for 24 h. The obtained extract was treated in the same manner as described for the Soxhlet method (see above).

Determination of plant extract yield

The percentage yield of the alkaloids was calculated as: % yield = $W_2 / W_1 \times 100$. Where W_2 is the weight of alkaloids crude extract and W_1 is the weight of the initial dried powder.

The crude extract of alkaloids was dissolved in dimethyl sulfoxide (DMSO 1%) to evaluate antioxidant activity.

Determination of antioxidant activity

The antioxidant activity of the alkaloid extracts of *M. pubescens* was determined using different tests.

Ferric reducing power (FRP)

The ferric reducing power was evaluated according to the method of Deepa et al. (2015). First, 2.5 mL of alkaloid extracts at various concentrations were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1 g/100 mL). The mixture was incubated at 50 °C. After 20 min, 2.5 mL of trichloroacetic acid (10 g/100 mL) was added. Then, 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1 g/100 mL). After 10 min, the absorbance was measured at 700 nm. The ferric reducing activity was expressed as gram ascorbic acid equivalent (AAE) per 100 g of dry weight (DW). Butylated hydroxyanisole (BHA), gallic acid and ascorbic acid were used as standards.

Total antioxidant capacity (TAC)

The total antioxidant capacity of the alkaloid extracts was determined by the phosphomolybdenum method of Prieto et al. (1999). A volume of 0.3 mL of each concentration was mixed with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixtures were incubated at 95 °C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm. The total antioxidant capacity was expressed as gram ascorbic acid equivalent (AAE) per 100 g of dry weight (DW). Ascorbic acid, gallic acid and trolox were used as positive controls.

DPPH radical scavenging activity

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity was performed according to the method of Jain et al. (2008.). 0.3 mL of alkaloid extracts was added to 2.7 mL of DPPH solution (60 μM). After 60 min of incubation, the absorbance was assessed at 517 nm. The percentage of DPPH radical scavenging activity was determined according to the formula: $\{[(Ac - At)/Ac] \times 100\}$, where Ac is the absorbance of the control and At is the absorbance of the extract. The extract concentration providing 50% antioxidant activity (IC₅₀ value (μg/mL)) was calculated from the graph of antioxidant activity percentages against alkaloid concentrations. BHA, gallic acid and ascorbic acid were used as positive controls.

ABTS radical scavenging activity

The ABTS (2, 2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging capacity was evaluated according to the method given by Re et al. (1999) with a little modification. In order to prepare the ABTS solution, an aliquot of 7 mM of ABTS was mixed with 2.45 mM of potassium persulfate and incubated 16 h at room temperature in the dark. The ABTS solution was diluted with ethanol until an absorbance of 0.70 ± 0.02 at 734 nm. 10 μL of alkaloids extract was added to 1 mL of ABTS solution. The absorbance was measured after 6 min at 734 nm. The percentage of ABTS radical scavenging activity of alkaloid extracts was determined from $\{[(Ac - At)/Ac] \times 100\}$, where Ac is the absorbance of the control and At is the absorbance of the extract. The decrease in absorption at 734 nm was used for calculating the IC₅₀ value (μg/mL). Trolox, BHA and gallic acid were used as positive controls.

β-Carotene bleaching (BCB) assay

The β -carotene bleaching inhibiting activity of *M. pubescens* alkaloids was evaluated using the β -carotene/linoleate model system (Moure et al., 2000). An amount of β -carotene (2 mg) was dissolved in 10 mL of chloroform. 1 mL of β -carotene solution was added to 20 μ L of linoleic acid and 200 mg of Tween 40 emulsifier. After total evaporation of the chloroform, 100 mL of distilled water saturated with oxygen was added. 0.2 mL of different concentrations of the alkaloids was mixed with 4 mL of this emulsion. The absorbance was immediately measured at 470 nm against a blank, consisting of the emulsion without β -carotene, which was considered at $t = 0$ min. After 2h of incubation at 50 °C in water bath, the absorbance was evaluated at 470 nm (A120). The inhibition of β -carotene bleaching percentage is determined by the following formula: $\{[(At(120) - Ac(120))/(Ac(0) - Ac(120))] \times 100\}$, Where At(120) is the absorbance of the sample at $t = 120$ min; AC(120) is the absorbance of the control at $t = 120$ min and AC(0) is the absorbance of the control at $t = 0$ min. The extract concentration providing 50% antioxidant activity (IC50 value (mg/mL)) was calculated from the graph of antioxidant activity percentage against alkaloid concentration. BHA, ascorbic acid and gallic acid were used as standard.

Hydroxyl radical scavenging activity

In this study the hydroxyl radical scavenging activity was assessed by two different tests:

Deoxyribose test

The hydroxyl free radical scavenging activity (HRSA) was evaluated according to the method of Halliwell et al. (1987), with some modifications. 1 mL of alkaloid extract was mixed with 0.1 mL of EDTA(1mM), 0.01 mL of chloride ferric (10 mM), 0.1 mL of hydrogen peroxide (10mM), 0.36 mL of deoxyribose (10 mM), 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1 mL of ascorbic acid (1mM) in sequence. After 1 hour of incubation at 37 °C, 1 mL of the mixture was added to 1 mL of thiobarbituric acid (1%) followed by 1 mL of trichloroacetic acid (0.5%). The mixtures were incubated in a water bath at 100 °C. After 20 min, the absorbance was measured at 532. The results were expressed as percentage of deoxyribose oxidation inhibition as determined by the following formula: $\{[(Ac - At)/Ac] \times 100\}$, where Ac is the absorbance of the control and At is the absorbance of the extract. IC50 value (mg/mL) calculated denote the concentration of the extract required to decrease the

absorbance by 50% at 532 nm. BHA, gallic acid and ascorbic acid were used as positive controls.

HOSC test

The HOSC (Hydroxyl radical scavenging capacity) method was tested according to the method of Moore et al. (2006). In a 96-well microplate, 170 μ L of fluorescein (9.28×10^{-8} M) and 40 μ L of H₂O₂ (0.1990 M) were added to 30 μ L of alkaloid extract. The reaction was started by adding 60 μ L of FeCl₃ solution (3.43 mM) at each well of the microplate. For 60 min at 37 °C, the fluorescence emitted by the reduced form of fluorescein was measured and recorded every 1 min. Emission and excitation wavelengths were 485 ± 20 nm and 530 ± 25 nm, respectively. Solution of sodium saline buffer (75 mM, pH 7.4) was used to prepare the fluorescein solution. Solutions of H₂O₂ and FeCl₃ were prepared in Milli-Q water. The trolox has been used as control standard at different concentrations (5, 10, 15, 20 and 30 μ mol/L). The HOSC values have been expressed using regression equation between the net area under the fluorescein decay curve and the trolox at different concentrations. The HORAC capacities were expressed as mmol of trolox equivalents (TE) per 100 g of dry weight (DW).

Oxygen radical absorbance capacity (ORAC)

The ORAC assay was evaluated according to the method of Huang et al. (2002), with some modifications. 25 μ L of alkaloid extract was mixed with 150 μ L of disodium fluorescein (2×10^{-7} mM). The mixture was incubated at 37 °C in the plate reader. After 10 min, 25 μ L of AAPH (2, 2'-azobis-2-methylpropanimidamide, dihydrochloride) (153 mM) was added. During 40 min at 37 °C, the fluorescence emitted by the reduced form of fluorescein was recorded every 1 min at 530 ± 25 nm excitation and 485 ± 20 nm emission wavelengths. Trolox at different concentration (5, 10, 20, 30 and 40 μ mol/L) was used as control standard. The solution of phosphate buffer saline (75 mM, pH 7.4) was used to prepare AAPH, fluorescein, and trolox solutions. The ORAC values have been expressed by applying the same equation used for the previous test. The ORAC capacities were expressed as mmol of trolox equivalents (TE) per 100 g of dry weight (DW).

Statistical analysis

Statistical analysis of the results was carried out with one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The comparison of these results

was taken at the probability of $P < 0.05$. All the data represent the average of three tests \pm standard deviation. Nonlinear regression test was used to determine the IC50. The correlation between all parameters was defined by Pearson correlation coefficient (r).

Results and discussion

Extract yields

A good extraction method must allow the complete extraction of the compounds of interest, avoiding any chemical modification. The extraction of bioactive compounds from plant materials using various solvent is an important step in obtaining phytochemical-rich product. The bioactive compounds from various plants have commonly been isolated by extraction methods such as Soxhlet extraction and maceration (Hayati et al., 2019).

In order to extract the highest alkaloid content from *M. pubescens*, extraction was carried out by applying two extraction techniques, maceration and Soxhlet extraction, using several solvents at different pH (alkalized chloroform, acidified methanol, neutral methanol and neutral ethanol). The results of this study show that the obtained extraction yields were affected by the extraction technique, nature and pH of solvent used (Figure 1). The highest extraction rates were obtained with ethanolic neutral (Soxhlet) and methanolic acidified (maceration) extracts with yields of $4.03 \pm 0.01\%$ and $4.02 \pm 0.01\%$, respectively, while the lowest extraction rate was found in the alkalized chloroform extract (maceration) with a value of $2.69 \pm 0.02\%$.

Using Soxhlet for the extraction, the results obtained showed that ethanol allowed to extract the highest rate of alkaloids compared to methanol. Concerning the extraction by maceration, it is the methanol which allowed to extract the highest rate in alkaloids that ethanol.

The extraction yield of plant extracts is affected strongly by the solvent, due to the presence of different compounds with different chemical characteristics and polarities. The variation between the extract yields was due to the polarities of different compounds present in the plant (Labiad et al., 2017).

The extraction yield using ethanol as solvent in Soxhlet extraction ($4.03 \pm 0.01\%$) was higher than that obtained using the same solvent for extraction by maceration ($3.32 \pm 0.01\%$), which indicates that the extraction technique influences the extraction yield of alkaloids. Djilani et al. (2006) demonstrated that the extraction technique used affects the extraction rate of alkaloids from the plants *Ruta graveolens* and *Hyoscyamus muticus*, which is consistent with the results of the present study.

Soxhlet extraction allows the sample to come into repeated contact with hot solvent, promoting the dissolution of the desired compounds. Extraction time and temperature are important parameters for optimizing extraction conditions (Galván D'Alessandro, 2013). This could explain the higher yield of alkaloids extracted by Soxhlet comparing to that extracted by maceration using the same extraction solvent. The differences observed between the alkaloid contents obtained by applying several extraction protocols could be explained by the presence of alkaloids of different nature and thus of solubility in the different extracts of *M. pubescens*.

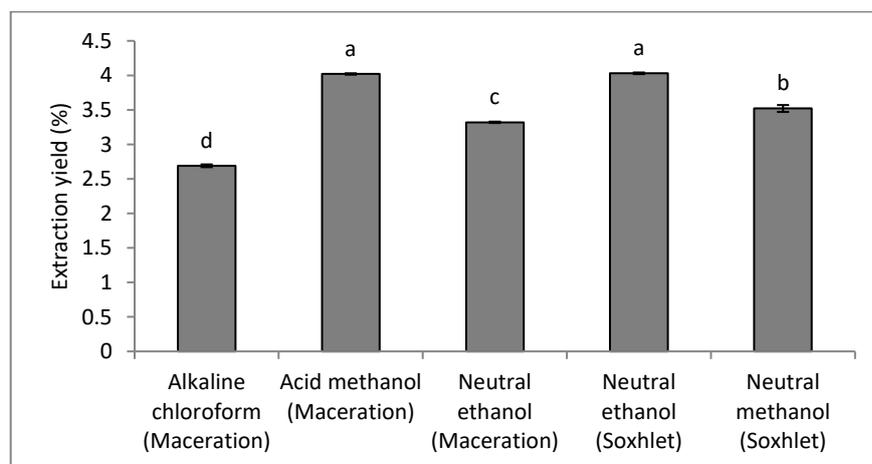


Figure 1. Effect of extraction technique, pH and solvent on the extraction yield of alkaloids from *M. pubescens*. Results with different letters are significantly different. All the data represent the average of three tests \pm standard deviation.

Authors have reported lower alkaloid contents than those found in this study. Contents ranging from 0.024 to 0.126 g/100 g, were extracted from two plants of the same family; *Artemisia judaica* and *Artemisia herba alba* (Elsharkawy and Shiboob, 2017). Different alkaloid fractions of *Fumaria officinalis* plant have given alkaloid contents ranging from 0.18 to 0.63 g/100 g (Khamtache-Abderrahim et al., 2016).

Antioxidant activities

Antioxidant compounds act through several mechanisms of action, including inhibition of free radical generation, enhancement of scavenging capacity against free radicals and reducing capacity. Thus, no test can accurately reflect the antioxidant effect of the compounds in a complex system. Therefore, to determine the antioxidant activity of compounds it is necessary to use the minimum two different methods (Antolovich et al., 2002). So, in the present study, the antioxidant activity of the alkaloid extracts of *M. pubescens* has been evaluated by various tests which determine the reducing activity of ferric and molybdate ions and the scavenging activity against DPPH, ABTS, hydroxyl and peroxy radicals. The extracts used to determine the antioxidant activity of *M. pubescens* are those which gave the best contents in alkaloids, and which are acidified methanol and alkalized chloroform extracts obtained by maceration and neutral ethanol extract obtained by Soxhlet. Ascorbic acid, gallic acid, trolox and butylated hydroxyanisole (BHA) were standards used as reference antioxidants.

Ferric reducing power (FRP)

The reducing properties of plant extracts are generally due to the presence of reducing compounds, which exert an antioxidant effect by giving a hydrogen atom which leads to the breaking of the radical chain. These reducing compounds also have the ability to react with some peroxide precursors, thus preventing peroxide formation (Govindan and Muthukrishnan, 2013). The results of this work showed that all alkaloid extracts of *M. pubescens* reduced ferric ions with significantly different values ($p < 0.05$), depending on the tested alkaloid concentration, extraction technique, pH and the used solvent (Figure 2). For all tested concentrations ranging from 5 to 25 mg/mL, statistical analysis of the results revealed that the strongest ferric reducing power was exerted by acid methanolic extract (maceration) with values ranging from 0.12 to 0.96 g AAE/100 g (DW). While the alkaline chloroform extract (maceration) gave the lowest reducing power, with values between 0.10 and

0.39 g AAE/100 g (DW). This study revealed that by increasing the concentration of alkaloids, the ferric reducing capacity of the extracts tested increases significantly ($p < 0.05$).

Ascorbic acid, gallic acid and BHA have been shown to have greater reductive capacities of ferric than those obtained with alkaloid extracts of *M. pubescens*, at different concentrations, with values between 2.04 and 5.09 g AAE/100 g, 0.71 and 1.88 g AAE/100 g, and between 1.20 and 3.62 g AAE/100 g, respectively.

Total antioxidant capacity (TAC)

The capacities of alkaloid extracts of *M. pubescens* to reduce molybdate were significantly different ($p < 0.05$), depending on the extraction protocol and the concentration of alkaloids tested. For alkaloid concentrations ranging from 5 to 25 mg/mL, the best total antioxidant capacity was obtained with the neutral ethanolic extract (Soxhlet), with contents between 0.32 and 0.88 g AAE/100 g (DW), while the lowest reducing activity of molybdate was determined with the chloroformic alkaline extract (maceration) with values ranging from 0.14 to 0.31 g AAE/100g (DW) (Figure 3). The statistical study did not reveal any significant difference ($p < 0.05$) between the total antioxidant capacities of the alkaloid extracts prepared by alkaline chloroform (maceration) and acid methanol (maceration) at concentrations ranging from 5 to 15 mg/mL, and they exhibited the weakest capabilities.

Total antioxidant capacity of the extracts of the studied plant is significantly ($p < 0.05$) proportional to the concentration of alkaloids for all tested extracts.

At concentrations ranging from 5 to 25 mg/mL, ascorbic acid, gallic acid and trolox tested in this activity showed a molybdate reducing capacity with values ranging from 1.53 to 2.82 g AAE/100 g, 1.36 to 1.82 g AAE/100 g and from 0.36 to 0.57 g AAE/100 g. These standards have shown greater molybdate reducing capacities than those obtained with alkaloid extracts of *M. pubescens* at different concentrations, except trolox.

DPPH radical scavenging activity

The evaluation of the DPPH radical scavenger activity revealed that the IC₅₀ values of the various alkaloid extracts of *M. pubescens* obtained showed significant differences ($p < 0.05$), depending on the extraction technique, nature and pH of solvent (Table 1). The neutral ethanolic extract (Soxhlet) exerted the largest DPPH radical scavenging effect with an IC₅₀ of 314 µg/mL, while the alkaline chloroform extract

(maceration) exerted the weakest scavenger effect with an IC₅₀ of 744.29 µg/mL.

BHA showed the highest DPPH radical scavenger activity than that exerted by all alkaloid extracts of the studied plant. However, alkaloid extracts obtained by neutral ethanol (Soxhlet) and acidified methanol (maceration) showed a significant ($p < 0.05$) DPPH radical scavenging activity superior to those exerted by gallic acid and ascorbic acid.

ABTS radical scavenging activity

The results of ABTS scavenger activity of *M. pubescens* extracts and of standards are shown in table number 1. Statistical analysis revealed the existence of

a significant difference ($p < 0.05$) between the tested alkaloid extracts according to the technique, pH and solvent. Acidified methanolic (maceration) and neutral ethanolic (Soxhlet) extracts exerted the most important ABTS radical scavenger effect, with IC₅₀ values of 370.83 and 395.81 µg/mL, respectively. While alkaline chloroform extract (maceration) showed the lowest scavenger activity with an IC₅₀ of 605.41 µg/mL.

The present work revealed that the alkaloid extracts obtained from *M. pubescens* exhibited lower ABTS radical scavenger activity than that expressed by trolox, BHA and gallic acid.

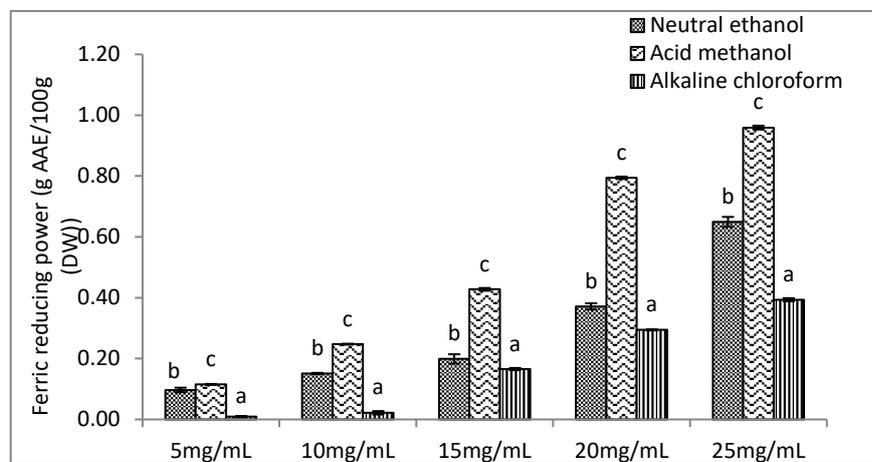


Figure 2. Effect of extraction technique, pH and solvent on ferric reducing power of *M. pubescens* alkaloids. Results with different letters are significantly different. All the data represent the average of three tests \pm standard deviation.

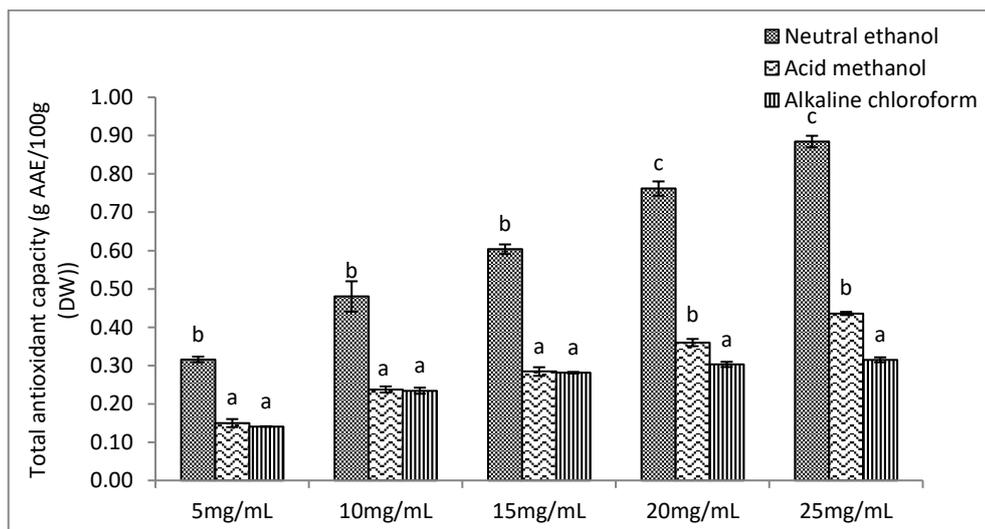


Figure 3. Effect of extraction technique, pH and solvent on total antioxidant capacity of *M. pubescens* alkaloids. Results with different letters are significantly different. All the data represent the average of three tests \pm standard deviation.

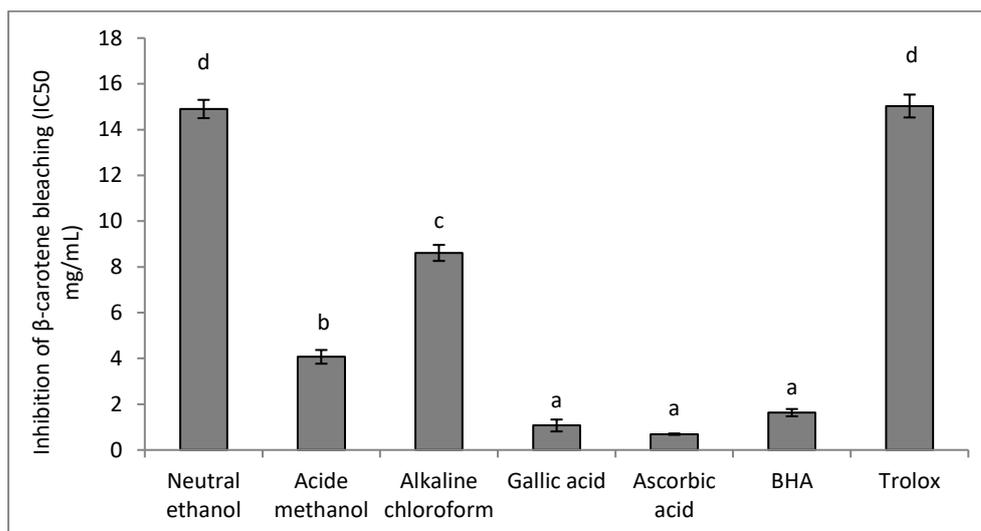


Figure 4. Effect of extraction technique, pH and solvent on β -carotene bleaching inhibiting activity of *M. pubescens* alkaloids. Results with different letters are significantly different. All the data represent the average of three tests \pm standard deviation.

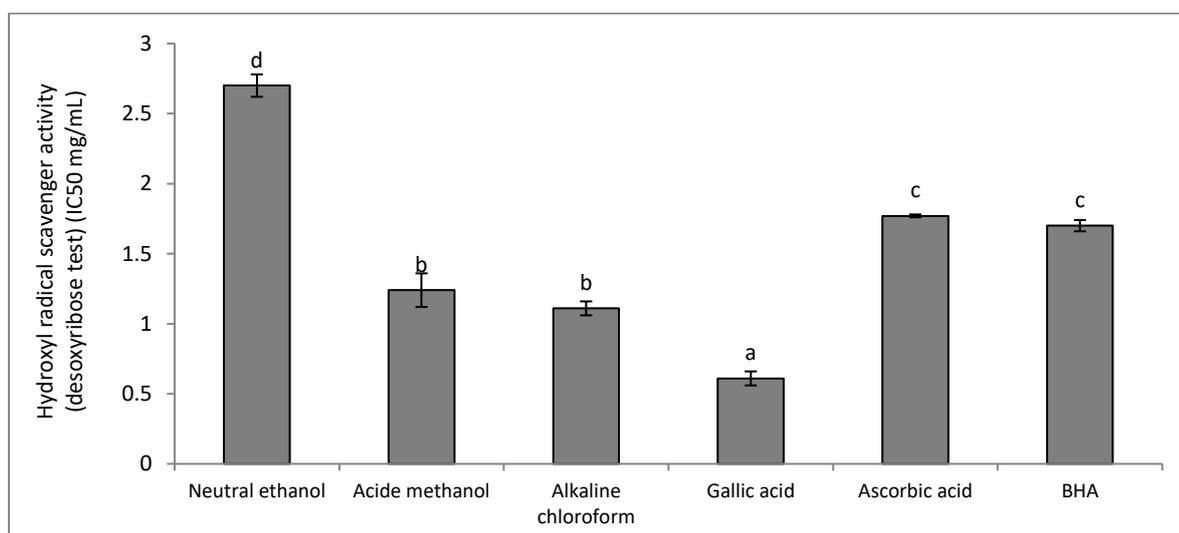


Figure 5. Effect of extraction technique, pH and solvent on hydroxyl radical scavenging activity determined by the deoxyribose test of *M. pubescens* alkaloids.

Results with different letters are significantly different. All the data represent the average of three tests \pm standard deviation.

β -carotene bleaching inhibiting (BCB) activity

The potential of the plant to inhibit lipid peroxidation was evaluated using β -carotene/linoleic acid bleaching test. β -carotene bleaching assay is considered among the oldest and most commonly applied methods of evaluating antioxidant activity of substances or extracts (Andrzej and Olszowy, 2010). In this test, the oxidation of linoleic acid in an aqueous emulsion system generates peroxy radicals. The break of π -conjugation by addition reaction of peroxy radical to a $C = C$ double bond of β -carotene, causes discoloration of the yellowish colour of the solution of β -carotene. The presence of antioxidants reduces the

extent of destruction of β -carotene by neutralizing free radicals derived from linoleic acid and therefore helps to prevent the oxidation and bleaching of β -carotene (Ueno et al., 2014).

The results of β -carotene bleaching inhibition by alkaloid extracts of *M. pubescens* presented significant differences ($p < 0.05$) according to the extraction technique, the pH and the nature of the solvent used (Figure 4). The acidic methanolic extract (maceration) revealed the most potent β -carotene bleaching inhibiting activity ($IC_{50} = 4.07$ mg/mL), while the alkaline chloroform extract (maceration) exhibited the lowest inhibiting activity ($IC_{50} = 14.90$ mg/mL).

This work has shown that gallic acid, ascorbic acid and BHA have shown the lowest β -carotene oxidation inhibiting activities than those exerted by the alkaloids of *M. pubescens*. However, trolox and neutral ethanolic extract exhibited the most important inhibitory activity compared to all other tested extracts.

Hydroxyl radical scavenging activity (HRSA) determined by deoxyribose test

In this test, the ability to trap the hydroxyl radical by plant extracts is based on Fenton reaction by measuring the generation of the OH• radical and its effect on the oxidation and degradation of biological molecules such as DNA deoxyribose. At low pH, hydroxyl radicals attack deoxyribose forming products that, upon heating with thiobarbituric acid (TBA), yield a pink chromogen. The presence of antioxidants protects deoxyribose and reduces chromogen formation (Halliwell et al., 1987).

Hydroxyl radical is the most reactive oxygen species, and has the shortest half-life compared with other ROS. The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipids, and proteins (Halliwell and Gutteridge, 1985). In this study, the results of the hydroxyl radical scavenger activity, determined by deoxyribose test, revealed that the IC50 values obtained show significant differences ($p < 0.05$), according to the technique, pH and solvent (Figure 5). The strongest hydroxyl radical scavenger activities were found using alkaline chloroform (maceration) and acidic methanol (maceration), with IC50 values of 1.11 and 1.24 mg/mL respectively. The neutral ethanolic extract (Soxhlet) exerted the weakest hydroxyl radical scavenger activity with an IC50 of 2.70 mg/mL.

The extracts prepared by alkaline chloroform and acid methanol showed the highest hydroxyl radical inhibitory activities than those exerted by all the standards tested, except gallic acid.

HOSC and ORAC capacities

Analysis of the results showed that acidic methanol extract exhibited the highest antioxidant activities among the tested extracts, thus HOSC and ORAC capacities were determined only on this extract (Figure 6). In the present study, the hydroxyl radical scavenging capacity was evaluated using also the HOSC assay (hydroxyl radical scavenging capacity). Hydroxyl radical is generated under physiological pH using a Fenton-like reaction with a definite end point. The HOSC assay is the best method compared to the deoxyribose assay widely used, because the HOSC

method generates more hydroxyl radicals (Karadag et al., 2009). This method combines both the percentage inhibition of oxidation reaction and the length over time of this inhibition in a single measurement. This study showed that the alkaloids extracted by acidic methanol exhibited a hydroxyl radical scavenging capacity (HOSC) with a value of 672.44 mmol TE/100 g (DW).

The ORAC (Oxygen radical absorbance capacity) method uses biologically relevant free radicals, integrates both time and degree of antioxidant activity into one data value, and it is readily adaptable to a high-throughput assay system. This method is based on the inhibition of the peroxy radical (ROO•) induced oxidation initiated by thermal decomposition of AAPH (2, 2'-azobis (2-amidino propane) dihydrochloride). The antioxidants react with the peroxy radicals and delay the degradation of fluorescein (Prior et al., 2003). The alkaloids extracted by acidic methanol exerted an ORAC capacity, with a value of 164.31 mmol TE/100 g (DW).

Analysis of the data of the antioxidant activities determined by the fluorimetric methods showed that the alkaloids of *M. pubescens* obtained by acidic methanol exhibited a HOSC activity higher than the ORAC activity. This indicates that the alkaloids of *M. pubescens* are more effective on the hydroxyl radical than on the peroxy radical.

Analysis of the results reveals that the antioxidant activities of alkaloid extracts of *M. pubescens* vary depending on the solvent and the technique used for the extraction and according to the method applied for the determination of antioxidant activity. This variation could be due to the quantity and/or the nature of the antioxidant substances present in the extracts studied. The antioxidant activity of the extracts was affected by the extraction solvent.

From the overall results of this study, the alkaloid extracts of *M. pubescens* exerted a reducing activity of ferric and molybdate ions. They also showed significant radical scavenger activity against DPPH, ABTS, hydroxyl, and peroxy radicals. These different antioxidant activities could be due to the structural diversity of the alkaloids present in extracts of *M. pubescens* and interactions between these compounds, which influence their mechanisms of action.

Numerous studies have reported that alkaloids extracted from plants have the ability to inhibit lipid peroxidation (Schmeda-Hirschmann et al., 2003; Khamtache-Abderrahim et al., 2016; Gan et al., 2017), to exert a radical scavenger activity against DPPH, ABTS (Liu et al., 2014; Gutiérrez et al., 2014; Dalimunthe et al., 2018). The reducing power of ferric and molybdate exerted by alkaloids has also been reported by other authors (Khamtache-Abderrahim et

al., 2016; Liu et al., 2014; Gan et al., 2017). Studies have shown the effectiveness of alkaloids in inhibiting other free radicals such as hydroxyl radical (Kiplimo et al., 2011; Gan et al., 2017; Darkwah et al., 2018) and peroxy radical (Tiong et al., 2013; Tian et al., 2018). These data indicate that the alkaloids can exert ferric and molybdate ion reducing activities and can inhibit DPPH, ABTS, hydroxyl and peroxy radicals. These results confirm the different antioxidant activities exerted by the alkaloid extracts of *M. pubescens*. *Correlation between alkaloids and antioxidant activities*

The correlation coefficients between the alkaloid concentrations of *M. pubescens* and the antioxidant activities obtained by several methods have been determined and are shown in Tables 2 and 3. The results of this work revealed the presence of a very highly significant correlation ($p < 0.001$) between the

alkaloid contents and the ferric and molybdate reducing activities. These results reflect the powerful contribution of *M. pubescens* alkaloids in these reducing activities. The study of the correlations between the reducing activities revealed a very significant correlation ($p < 0.01$) between the ferric reducing power (FRP) and the molybdate reducing activity (TAC).

A very highly significant correlation ($p < 0.001$) was established between the DPPH and ABTS scavenger activities. However, the correlation between the hydroxyl radical scavenger activity (deoxyribose test) (HRSA) and β -carotene bleaching inhibiting activity (BCB) is highly significant ($p < 0.01$). These results indicate that the alkaloids of *M. pubescens* have a good capacity to trap: DPPH, ABTS, hydroxyl, and peroxy radicals. The study showed insignificant correlations between DPPH, ABTS and HRSA, BCB.

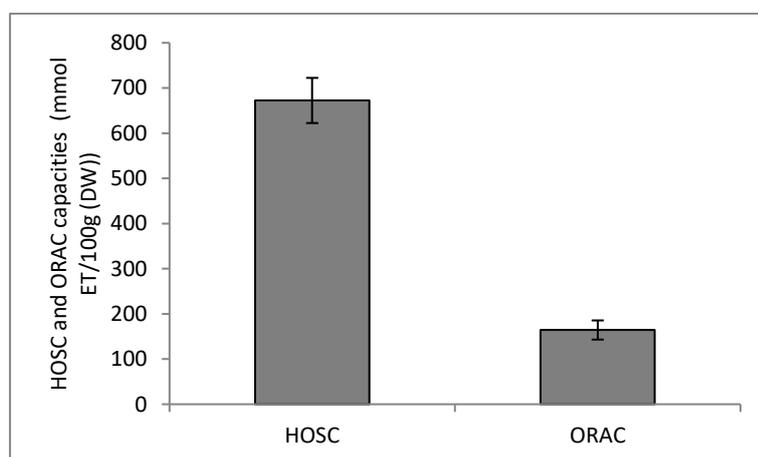


Fig 6. Effect of *M. pubescens* alkaloids on hydroxyl radical scavenging and oxygen radical absorbance capacities. All the data represent the average of three tests \pm standard deviation

Table 2. Correlation matrix between alkaloid contents and reducing activities of ferric and molybdate ions

	Alkaloids	FRP	TAC
Alkaloids	1		
FRP	0.80 ***	1	
TAC	-0.58***	0.44**	1

* $p < 0.05$, significant correlation. ** $p < 0.01$, very significant correlation. *** $p < 0.001$, extremely significant correlation

Table 3. Correlation matrix between radical scavenging activities of *M. pubescens* alkaloids

	DPPH	ABTS	HRSA	BCB
DPPH	1			
ABTS	0.93 ***	1		
HRSA	-0.6	-0.42	1	
BCB	-0.22	0.01	0.73**	1

* $p < 0.05$, significant correlation. ** $p < 0.01$, very significant correlation. *** $p < 0.001$, extremely significant correlation.

These results show that the bioactive substances responsible for each activity are not the same. Few studies have been done on establishing correlations between alkaloid levels and antioxidant activities. Gan et al. (2017) reported relationship between the ferric reducing capacity, the hydroxyl radical scavenger activity, the lipid peroxidation inhibition ability and alkaloids. The antioxidant activity of extract depends on the antioxidant contents, the structure and interaction between these antioxidant compounds (Rice-Evans et al., 1997; Bourgou et al., 2008). The antioxidant activities of the individual compounds may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features (Patt et al., 1990). Due to their redox properties, antioxidants act as hydrogen donors, reducing agents, and singlet and triplet oxygen quenchers (Pietta, 2000).

Conclusions

This present work is the first study to evaluate the effect of the extraction method and solvent on alkaloids content of *M. pubescens* and their antioxidant activity. No study has determined the antioxidant activity of alkaloids of the plant. Based on the above results, it can be concluded that the extraction technique, pH and nature of extraction solvent have a significant effect on the alkaloids content and the antioxidant activity. In addition, the results of the antioxidant activity determined by several tests have shown that the alkaloid extracts of *M. pubescens* exerted a reducing activity of ferric and molybdate ions and showed significant radical scavenger activity against DPPH, ABTS, hydroxyl, and peroxy radicals. The richness in alkaloids and the various antioxidant activities of *M. pubescens* observed in this work could justify its traditional use. It would be interesting to characterize the alkaloids of this plant and to study their biological activities.

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References

- Andrzej, L., Olszowy, D.M. (2010): cInfluence of some experimental variables and matrix components in the determination of antioxidant properties by β -carotene bleaching assay: experiments with BHT used as standard antioxidant, *Eur. Food Res. Technol.* 231, 835–840. <https://doi.org/10.1007/s00217-010-1333-4>.
- Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S., Robards, K. (2002): Methods for testing antioxidant activity, *Analyst.* 127, 183–198. <https://doi.org/10.1039/B009171P>.
- Bargagli, E., Olivieri, C., Bennett, D., Prasse, A., Muller-Quernheim, J., Rottoli, P. (2009): Oxidative stress in the pathogenesis of diffuse lung diseases: A review, *Respir. Med.* 103, 1245–1256. <https://doi.org/10.1016/j.rmed.2009.04.014>
- Bourgou, S., Ksouri, R., Bellila, A., Skandrani, I., Falleh, H., Marzouk, B. (2008): Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots, *C. R. Biol.* 331, 48–55. <https://doi.org/10.1016/j.crv.2007.11.001>
- Bruneton, J. (2009): Pharmacognosie–Phytochimie, plantes médicinales, 4e éd., Tec & Doc– Éditions médicales internationales, Paris, pp 937– 954.
- Dalimunthe, A., Hasibuan, P.A., Z, Silalahi, J., Sinaga, S.F., Satria, D. (2018): Antioxidant Activity of Alkaloid Compounds from *Litsea cubeba* Lour, *Orient. J. Chem.* 34(2), 1149–1152. <https://doi.org/10.13005/ojc/340270>
- Darkwah, W.K., Ao, Y., Adinortey, M.B., Weremfo, A., Abrokwah, F.K., Afriyie, E. (2018): Total phenolic, flavonoid and alkaloid contents, oxidative DNA damage protective and antioxidant properties of methanol and aqueous extracts of *Dissotis rotundifolia* whole plant, *Free radic. antioxid.* 8(2). <https://doi.org/10.5530/fra.2018.2.13>
- Deby–Dupont, G., Deby, C., Lamy, M. (2002): Données actuelles sur la toxicité de l’oxygène, *Réanimation* 11, 28–39. [https://doi.org/10.1016/S1624-0693\(01\)00208-0](https://doi.org/10.1016/S1624-0693(01)00208-0)
- Deepa, M., Darsan, M.B., Ramalinga, C. (2015): In Vitro Evaluation of the antioxidant, anti-inflammatory and antiproliferative activities of the leaf extracts of *Excoecaria agallocha* L, *Int. J. Pharm. Sci.* 7 (11), 346–52.
- Djellouli, M., Moussaoui, A., Benmehdi, H., Ziane, L., Belabbes, A., Badraoui, M., Slimani, N., Hamidi, N. (2013): Ethnopharmacological study and phytochemical screening of three plants (Asteraceae family) from the region of south west Algeria, *Asian J. Appl. Sci.* 2 (2), 159–165.
- Djilani, A., Legseir, B., Soulimani, R., Dicko, A., Younos, C. (2006): New Extraction Technique for Alkaloids, *J. Braz. Chem. Soc.* 17(3), 518–520. <https://doi.org/10.1590/S0103-50532006000300013>
- Elsharkawy, E.R., Shiboob, M.H. (2017): Antioxidant Activity of Phenolic and Alkaloid Fractions Accumulated in *Artemisia Judaica* and *Artemisia*

- Herba Alba*, *J. Nat. Remedies*. 17 (4), 154–164. <https://doi.org/10.18311/jnr/2017/18731>
- Galván D'Alessandro, L. (2013): *Eco-procédés pour la récupération sélective d'antioxydants à partir d'Aronia melanocarpa et ses co-produits*. Université Lille 1.
- Gan, J., Feng, Y., He, Z., Li, X., Zhang, H. (2017): Correlations between Antioxidant Activity and Alkaloids and Phenols of Maca (*Lepidium meyenii*), *J. Food Qual.* 2017. <https://doi.org/10.1155/2017/3185945>
- Govindan, P., Muthukrishnan, S. (2013): Evaluation of total phenolic content and free radicals scavenging activity of *Boerhavia erecta*, *J. Acute Med.* 3 (2013), 103–109. <https://doi.org/10.1016/j.jacme.2013.06.003>
- Gutiérrez, A.D.M., Bah M, Garduño, R. M. L., Mendoza, D. S.O., Serrano, C., V. (2014): Anti-inflammatory and antioxidant activities of methanol extracts and alkaloid fractions of four mexican medicinal plants of solanaceae, *Afr J Tradit Complement Altern Med* 11(3), 259–267. <https://doi.org/10.4314/ajtcam.v11i3.36>
- Halliwell B, Gutteridge JMC. (1985): Free radicals ageing and disease, free radicals in biology and medicine [M]. 2nd ed. Oxford, UK: Clarendon Press. 279–315.
- Halliwell, B., Gutteridge, J.M. (1990): Role of free radicals and catalytic metal ions in human disease: an overview, *Meth. Enzymol.* 186, 1–85. [https://doi.org/10.1016/0076-6879\(90\)86093-B](https://doi.org/10.1016/0076-6879(90)86093-B)
- Halliwell, B., Gutteridge, J.M.C., Arnoma, O.L. (1987): The deoxyribose method: A simple test tube assay for the determination of rate constant for reaction of hydroxyl radical, *Anal. Biochem.* 165, 215–219. [https://doi.org/10.1016/0003-2697\(87\)90222-3](https://doi.org/10.1016/0003-2697(87)90222-3)
- Hayati, E.K., Madjid, A.D.R., Yuliani, D., Safitri, E.W., Rahmah, F.T., Qoriati, Y. (2019): Optimization of ultrasound-assisted extraction of alkaloids from *Acalypha indica*: Solvent and extraction time variation, *AIP Conf Proc.* 2120, 080026; <https://doi.org/10.1063/1.5115764>
- Huang, D., Ou, B., Hampsch–Woodill, M., Flanagan, J.A., Prior, R.L. (2002): High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96–well format, *J. Agric. Food Chem.* 50 (16), 4437–4444. <https://doi.org/10.1021/jf0201529>
- Huet, O., Duranteau, J. (2008): Dysfonction endothéliale: rôle des radicaux libres Endothelial dysfunction: Involvement of reactive oxygen species, *Réanimation* 17, 387–392. <https://doi.org/10.1016/j.reaurg.2008.03.020>
- Jain, A., Soni, M., Deb, L., Jain, A., Rout, S., Gupta, V., Krishna, K. (2008): Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. Leaves, *J. Ethnopharmacol.* 115(1), 61–66. <https://doi.org/10.1016/j.jep.2007.09.009>
- Karadag, A., Ozcelik, B., Saner, S. (2009): Review of Methods to Determine Antioxidant Capacities, *Food Anal. Methods.* 2, 41–60. <https://doi.org/10.1007/s12161-008-9067-7>
- Khamtache–Abderrahima, S., Lequart–Pillon, M., Gontier, E., Gaillard, I., Pilard, S., Mathiron, D., Djoudad–Kadji, H., Maiza–Benabdesselam, F. (2016): Isoquinoline alkaloid fractions of *Fumaria officinalis*: Characterization and evaluation of their antioxidant and antibacterial activities, *Ind Crops Prod.* 94, 1001–1008. <https://doi.org/10.1016/j.indcrop.2016.09.016>
- Kiplimo, J.J., Islam, Md., Koorbanally, N.A. (2011): A Novel Flavonoid and Furoquinoline Alkaloids from *Vepris glomerata* and their Antioxidant Activity, *Nat. Prod. Commun.* 6 (12). <https://doi.org/10.1177/1934578X11100601215>
- Koehlin–Ramonatxo, C. (2006): Oxygène, stress oxydant et supplémentations antioxydantes ou un aspect différent de la nutrition dans les maladies respiratoires, *Nutr. Clin. Metab.* 20, 165–177. <https://doi.org/10.1016/j.nupar.2006.10.178>
- Labiad, M.H., Harhar, H., Ghanimi, A., Tabyaoui, M. (2017): Phytochemical screening and antioxidant activity of moroccan *Thymus satureioides* extracts, *J. Mater. Environ. Sci.* 8 (6), 2132–2139. <https://doi.org/10.36349/easjpp.2022.v04i05.003>
- Liu, C.M., Kao, C.L., Wu, H.M., Li, W.J., Huang, C.T., Li, H.T., Chen, C.Y. (2014): Antioxidant and Anticancer Aporphine Alkaloids from the Leaves of *Nelumbo nucifera* Gaertn. cv. Rosa–plena, *Molecules* 19, 17829–17838. <https://doi.org/10.3390/molecules191117829>
- Maiza, K., Brac de la Perrière, R.A., Hammiche, V. (1995): Pharmacopée traditionnelle saharienne, *Revue de Médecines et Pharmacopées Africaines* 9 (1), 71–75.
- Metrouh–Amir H., Amir, N. (2018): Evaluation in vivo of anti-inflammatory and analgesic properties of *Matricaria pubescens* alkaloids, *S. Afr. J. Bot.* 116, 168–174. <https://doi.org/10.1016/j.sajb.2018.03.008>
- Metrouh–Amir, H., Duarte, C.M.M., Maiza, F. (2015): Solvent effect on total phenolic contents, antioxidant, and antibacterial activities of *Matricaria pubescens*, *Ind Crops Prod.* 67, 249–256. <https://doi.org/10.1016%2Fj.indcrop.2015.01.049>
- Moore, J., Yin, J., Yu, L. (2006): Novel fluorometric assay for hydroxyl radical scavenging capacity (HOSC) estimation, *J. Agric. Food Chem.* 54 (3), 617–626. <https://doi.org/10.1021/jf052555p>
- Moure, A., Franco, D., Sineiro, J., Dominguez, H., Nunez, M.J., Lema, J.M. (2000): Evaluation of extracts from *Gevuina avellana* hulls as antioxidants, *J. Agric. Food Chem.* 48 (9), 3890–3897. <https://doi.org/10.1021/jf000048w>
- Mukherji, SM., Singh, SP. (1986): Reaction mechanism in organic chemistry. Madras: Macmillan India Press. 1986.
- Patt, D.E., Hudson, B.J.F. (1990): Natural antioxidants not exploited commercially. In: food antioxidants. Hudson B.J.F., Ed: Elsevier Applied Science: London, U. K. 171–191.

- Pietta, P.G. (2000): Flavonoids as antioxidants, *J. Nat. Prod.* 63, 1035–1042. <https://doi.org/10.1021/np9904509>
- Prieto, P., Pineda, M., Aguilar, M. (1999): Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal. Biochem.* 269, 337–341. <https://doi.org/10.1006/abio.1999.4019>
- Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., et al. (2003): Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples, *J. Agric. Food Chem.* 51, 3273–3279. <https://doi.org/10.1021/jf0262256>
- Qazi, M.A., Molvi, K.I. (2018): Free Radicals and their Management, *Am J Pharm Health Res.* 6(04), 1–10. <https://doi.org/10.46624/ajphr.2018.v6.i4.001>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice–Evans, C. (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* 26, 1231–1237. [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3)
- Rehman, S., Khan, H. (2017): Advances in Antioxidant Potential of Natural Alkaloids, *Curr. Bioact. Compd.* 13 (2), 101–108. <https://doi.org/10.2174/1573407212666160614075157>
- Rice–Evans, C.A., Sampson, J., Bramley, P.M., Holloway, D.E. (1997): Why do we expect carotenoids to be antioxidants in vivo?, *Free Radic. Res.* 26, 381–398. <https://doi.org/10.3109/10715769709097818>
- Roberts, C.K., Sindhu, K.K. (2009): Oxidative stress and metabolic syndrome, *Life Sci.* 84, 705–712. <https://doi.org/10.1016/j.lfs.2009.02.026>
- Schmeda–Hirschmann, G., Rodriguez, J.A., Theodulou, C., Astudillo, S.L., Feresin, G.E., Tapia, A. (2003): Free radical scavengers and antioxidants from *Peumus boldus* Mol. (Boldo), *Free Radic. Res.* 37, 447–452. <https://doi.org/10.1080/1071576031000090000>
- Suau, R., Cabezudo, R., Rico, R., Najera, F., Lopez–Romero, J.M. (2002): Direct determination of alkaloid contents in *Fumaria* species by GCMS, *Phytochem. Anal.* 13, 363–367. <https://doi.org/10.1002/pca.669>
- Tian, W., Zhi, H., Yang, C., Wang, L., Long, J., Xiao, L., Liang, J., Huang, Y., Zheng, X., Zhao, S. et al. (2018): Data on chemical composition of alkaloids of *Plumula nelumbinis* and antioxidant activity from thirteen habitats in China, *Data Br.* 21 (2018), 1591–1597. <https://doi.org/10.1016/j.dib.2018.11.004>
- Tiong, S.H., Looi, C.Y., Hazni, H., Aditya Arya, A., Paydar, M.J., Wong, W.F., Cheah, S.C., Mustafa, M.R., Awang, J. (2013): Antidiabetic and Antioxidant Properties of Alkaloids from *Catharanthus roseus* (L.) G. Don, *Molecules* 18, 9770–9784. <https://doi.org/10.3390/molecules18089770>
- Ueno, H., Yamakura, S., Arastoo, R.S., Oshima, T., Kokubo, K. (2014): Systematic Evaluation and Mechanistic Investigation of Antioxidant Activity of Fullerenols Using β -Carotene Bleaching Assay, *J. Nanomater.* 2014, 1–7. <https://doi.org/10.1155/2014/802596>
- Yan, D., Jin, C., Xiao, X.H., et al. (2008): Antimicrobial Properties of Berberine Alkaloids in *Coptis Chinensis* Franch by Microcalorimetry, *J. Biochem. Biophys. Methods.* 70 (6), 845–849. <https://doi.org/10.1016/j.jbbm.2007.07.009>
- Yubin, J.I., Miao, Y., Bing, W., Yao, Z. (2014): The extraction, separation and purification of alkaloids in the natural medicine, *J. Chem. Pharm. Res.* 6(1), 338–345. <http://jocpr.com/vol6-iss1-2014/JCPR>