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Original Research Article

Antioxidant and Anti-Inflammatory Activities of the Volatile Components of *Tropaeolum Majus* L. (Garden Nasturtium) From North Western Algeria

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ABSTRACT

The current study was undertaken to investigate the antioxidant and anti-inflammatory activities of the volatile components of the aerial parts of Tropaeolum majus L. The fresh aerial parts of T. majus were extracted using Clevenger-type apparatus according to the European Pharmacopoeia. The volatiles were then recovered with diethyl ether. Antioxidant capacity was assessed by in vitro tests using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assay. The volatiles showed strong activity against the radical DPPH, compared with ascorbic acid. In FRAP assay the ability of volatile fraction to reduce ferric ions was determined. The volatile components were also tested against 5-lipoxygenase (5-LO) and 12-lipoxygenase (12-LO) inhibition. These components seem to be more active against 5-LO than 12-LO. The benzyl isothiocyanate, the major identified component, alone is a strong 5-LO inhibitor. The obtained results indicate the possibility of exploitation of volatile components of T. majus for a new source of nutraceutical foods.

Keyword: *Tropaeolum majus* L.; volatiles; antioxidant; anti-inflammatory

INTRODUCTION

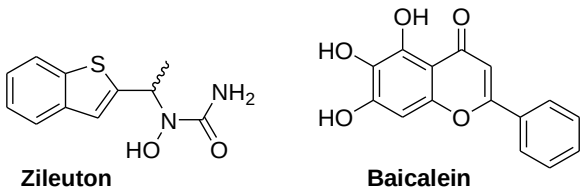
Free radicals are known to be the major cause of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer [1] and can cause cellular injuries and initiate peroxidation of polyunsaturaed fatty acids in biological membranes. The tissue injury caused by reactive oxygen species (ROS) may include

DNA [2] and protein damage, and oxidation of important enzymes in the human body [3]. Recently, many fruits, herbs and vegetables are potentially useful for decreasing the risks of several chronic diseases. Flavonoids, tannins, anthocyanins and other phenolic constituents present in plants are potential antioxidants [4]. In addition, numerous studies have demonstrated that essential oils obtained from plants have therapeutic uses in human medicine due to their anticancer, antinociceptive, antiphlogistic, antiviral, antibacterial and antioxidant properties [5]. The use of natural products isolated from medicinal plants represents a good source of novel and clinically important drugs in connection with the treatment of some kinds of clinical disorders [6].

Lipoxygenases are a family of monomeric non-heme, non-sulfur iron dioxygenases, which catalyze the conversion of polyunsaturated fatty acids into conjugated hydroperoxides. Polyunsaturated fatty acids are important components of lipid membranes as well as substrates for the biosynthesis of signaling molecules such as prostaglandins, leukotrienes and endocannabinoids [7]. Lipoxygenases are found in plants, fungi and animals and their roles in some of these species have been extensively studied and reviewed [7, 8].

Mammalian lipoxygenases have been extensively studied with a number of reports describing functions and properties of various lipoxygenase isozymes in these systems. In human cells six lipoxygenase isoforms have been described and sequenced and a number of biological roles have been described for these enzymes [7, 8].

The inflammatory response is necessary for the host defense and wound healing. The 5-lipoxygenase (5-LO) and 12-lipoxygenase (12-LO) pathways play a central role in the response by generating inflammatory mediators. However, uncontrolled and unregulated production of these mediators can contribute to chronic and acute pathologies such as asthma, atherosclerosis, arthritis and cancer [9, 10]. Among known inhibitors of 5-LO, only the anti-asthmatic drug Zileuton (Figure 1) is approved for human use as the racemic mixture [11]. However, undesirable hepatotoxicity as well as its pharmacokinetic profile requiring frequent dosing limits its usefulness in treatments [11-13]. Baicalein (Figure 1), the major component in the root of *Scutellaria baicalensis*, has been shown to induce apoptosis in breast, prostate, colon, and pancreatic cancer cell lines [14-17]. As reported in the literature, the potency of baicalein is thought to be due to the selective inhibition of platelet 12-LO [18-24].



Zileuton **Baicalein**

Fig.1: Structures of Zileuton and Baicalein

Therefore, the regulation of the 5-LO and 12-LO pathways has been subjected to numerous studies involving natural compounds in recent years [25, 26]. Given the numerous beneficial properties that exert *T. maius*, we evaluate its potential inhibition of the 5-LO and 12-LO pathways. Thus, this study evaluates the antioxidant activity and anti-inflammatory properties of the volatiles of T. majus.

MATERIALS AND METHODS

Plant materials

The aerial parts (orange flowers, stems, leaves) of *T. majus* were collected in Nedroma [Latitude:

34°52'N; Longitude: 1°14'O; Altitude: 650m] during the flowering period (May 2012). Botanical identification of plant was conducted by Prof. Noury BENABADJI and a voucher specimen of the plant was deposited in the Herbarium of the Laboratory of Biology, Abou bekr Belkaïd University, Tlemcen, Algeria.

Plant extracts preparation

Volatile components (VCs) were obtained from the cooling of steam in the hydrodistillation process [27]. Practically, the volatile components were obtained by simple hydrodistillation (5 hours) using about five\fold water to the fresh weight of the plant material. They were recovered with diethyl ether (1 mL). The organic layer was dried by anhydrous sodium sulfate and then evaporated at room temperature [28]. The volatiles were then stored at +4°C.

Antioxidant activity

Radical-scavenging capacity of volatile components

The antioxidant activity *in vitro* was evaluated by the measurement of the capacity of trapping of radical 1,1-diphenyl-2-picrylhydrazil (DPPH), according to the method described in literature [29-31] with slight modifications. Fifty microliters of various concentrations of the essential oil in ethanol was added to 5 mL of a 0.004% ethanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of the free radical by DPPH in percent (I%) was calculated in following way:

$I\% = 100 \times (A_{blank} - A_{sample}) / A_{blank}$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The actual decrease in absorbance induced by the tested sample (change of color deep-violet to light yellow) was compared to that of the positive control ascorbic acid (AA). The IC_{50} value represented the concentration of volatile compounds that causes 50% inhibition was calculated from the linear regression algorithm of the graph plotted inhibition percentage against extract concentration. Experiments were carried out in triplicate and the mean value was recorded.

Ferric reducing antioxidant power assay (FRAP)

The reducing power assay was conducted as described by Oyaizu [32], ascorbic acid (AA) was used as the positive control. Different concentrations of volatile constituents of fresh *T. majus* (0.019-0.72 mg/mL) in 1 mL of methanol were mixed with equal volume of phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with (2.5 mL) of distilled water and (0.5 mL) of ferric chloride (0.1%) and the absorbance of the resulting solution was measured at 700 nm. Increased absorbance of the reaction mixture interprets as an increase in reducing the power of sample.

Anti-inflammatory activity

12-lipoxygenase assay

Blood was obtained from healthy human volunteers (citrate as anticoagulant) following written consent under an approved institutional review board protocol (Comité d'éthique de la recherche avec les êtres humains, Université de Moncton). Blood was centrifuged at 250 x *g* for 10 minutes. Platelet-rich plasma (PRP) was obtained and centrifuged at 400 x *g* for 2 minutes to remove contaminating erythrocytes. The PRP was then centrifuged at 1300 x *g* for 10 minutes. The pellets containing platelets were resuspended in Tyrode's Buffer pH 7.4 (134 mM NaCl, 2.9 mM KCl, 0.34 mM Na_2HPO_4, 12 mM $NaHCO_3$, 20 mM HEPES, 1 mM $MgCl_2$, 5 mM glucose, 0.5 mg/mL solution of Bovine Serum Albumin (BSA), 5 mM $CaCl_2$) at 1 x 10^8 cells/mL. Compounds were then incubated in the presence of platelets (5 x 10^7

cells) at the indicated concentrations for 5 minutes at 37°C. The generation of 12-lipoxygenase products was initiated by adding 10 µM of arachidonic acid (Cayman Chemicals) and 0.5 U/mL of thrombin (Sigma-Aldrich) followed by a 15 minutes incubation at 37°C. Reaction was stopped by addition of two volumes of cold MeOH:MeCN (1:1) and 50 ng of Prostaglandin B2 (PGB_2) as internal standard. Samples were then stored at -20°C until processing and separation on RP-HPLC with diode array detection as previously described [33] for quantification of the 12-lipoxygenase product 12-hydroxyeicosatetraenoic acid (12-HETE).

5-lipoxygenase assay

Blood (heparin as anticoagulant) was obtained as described above. Human polymorphonuclear neutrophils (PMN) were then isolated as described previously [34]. Briefly, blood was centrifuged at 250 x *g* for 10 minutes and PRP was discarded. Erythrocyte removal was performed by dextran sedimentation. PMNs were obtained following centrifugation on a lymphocyte separation medium cushion (density of 1.077 g/mL, Wisent) at 900 x *g* for 20 minutes at room temperature. 5-lipoxygenase assay was then performed as described previously [35]. Briefly, PMNs (10^7 cells/mL) in Hank's Balance Salt Solution (HBSS, Wisent) supplemented with 1.6 mM $CaCl_2$ and 0.4 U/mL adenosine deaminase (Sigma-Aldrich) were pre-incubated with the test compounds for 5 minutes at 37°C. Stimulation was initiated with the addition of 10 µM of arachidonic acid (Cayman Chemicals) and 1 µM of thapsigargin (Sigma-Aldrich). The reaction was stopped and samples were separated by RP-HPLC with diode array detection as indicated above with quantification of 5-lipoxygenase products performed by mesuring the sum of leukotriene B_4, its trans isomers, 20-COOH- and 20-OH-leukotriene B_4 and 5-hydroxyeicosatetraenoic acid.

RESULTS AND DISCUSSION

Antioxidant power was measured through two *in-vitro* methods including: 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay; and ferric reducing antioxidant power (FRAP). Figure 2 demonstrates DPPH scavenging activity, expressed in percentage, caused by different concentration of volatiles. Figure 3 presents the dose dependent ferric reducing powers of the volatiles and ascorbic acid (AA).

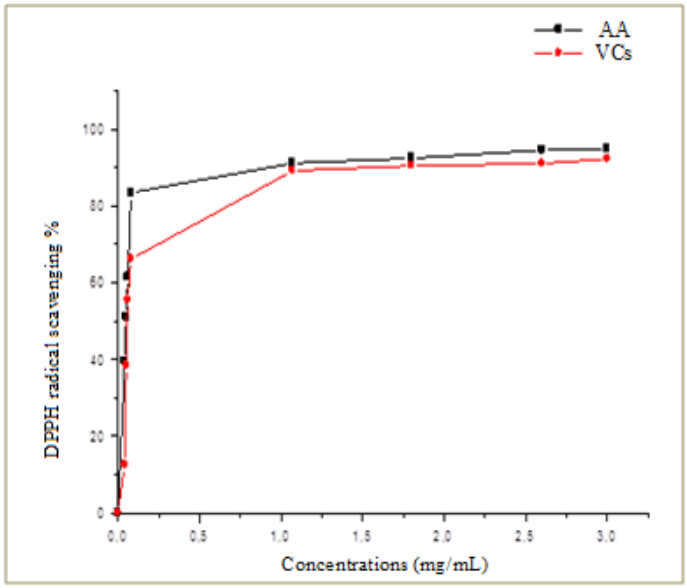


Fig. 2: DPPH reduction measured after 30 min of reaction as function of concentration of volatiles extracted from the aerial parts of *T. majus*

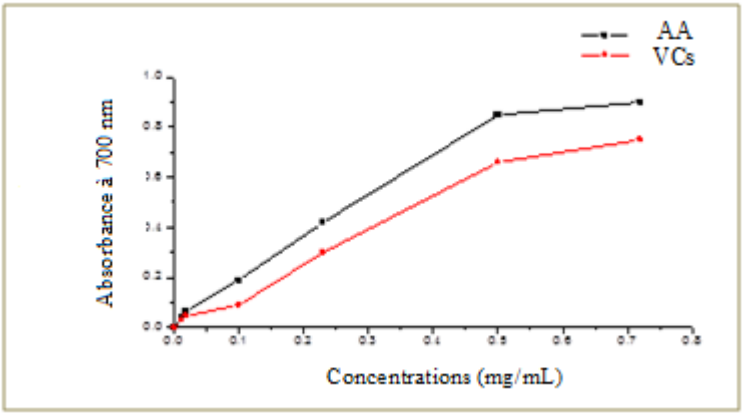


Fig. 3: Reducing power of AA and of volatiles extracted from the aerial parts of *T. majus*

The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts [29, 36]. It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used. The two methods DPPH and FRAP chosen are the most commonly used for the determination of antioxidant activities of plant extracts and/or volatile fraction. The reduction ability of DPPH radicals formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [29]. The results obtained during the test of measurement of inhibition percentage of radical DPPH show that the inhibition percentage of the free radical increases with the increase in the concentration of volatiles or AA. It was also noticed that the inhibition percentage of the free radical for volatiles components is lower than of AA for all concentrations used. For a concentration of 3mg/mL, volatiles reveal an inhibition percentage of 92.3%, while AA is inhibited with 94.8% of DPPH. The free radical scavenging activity is usually presented by the IC_{50} value. Concentrations of the antioxidant providing 50 % inhibition of DPPH in the test solution (IC_{50}) was determined from plotted graph of scavenging activity against the different concentrations of volatiles of *T. majus* and AA. The results show that the volatiles compounds and AA were able to reduce the stable free radical DPPH with an IC_{50} of 0.06 and 0.045 mg/mL, respectively. From these results, volatiles exhibited similar radical scavenging potential compared to the standard used and those cited in literature: quercetin (IC_{50}: 0.06 mg/mL) and luteolin (IC_{50}: 0.08 mg/mL) [37]. It should be noted that this good activity depends on the concentration of samples and it can be due to the high percentage of main constituents and to synergy among them.

The reducing power of the volatiles of T. majus was measured under the FRAP assay. This assay is based on the ability of antioxidants to reduce the ferric form (Fe^{3+}) to the ferrous form (Fe^{2+}). Prussian blue colored complex is formed by adding $FeCl_3$ to the ferrous (Fe^{2+}) form. Therefore, reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm [30] and [31]. In this assay, yellow color of the test solution changes to green or blue color depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric reducing power. Increasing absorbance indicates an increase in reductive ability. The reducing power of volatiles components and AA increased with increasing concentration. The results show that volatile of T. majus is a good antioxidant

agent. The present assay confirms the results obtained from DPPH.

Inflammatory lipid mediators are generated in part by the 5-LO and 12-LO pathways [9, 10]. These mediators play an important role in the everyday host defense. However, uncontrolled overproduction of these mediators has been linked to several pathologies such as asthma, atherosclerosis, arthritis and cancer [9, 10]. Therefore, we evaluated the ability of the volatile components of the aerial parts of *T. majus* L. (VCs) to affect the 5-LO and 12-LO pathways, subsequently reducing the amount of inflammatory lipid mediators generated. Benzyl isothiocyanate (Bzlso), the identified major component of the aerial parts [38], was also tested. In a dose-response experiments, VC and Bzlso inhibited the generation of 5-LO products (Figure 4). At 7.5µg/mL VCs inhibited 5-LO product biosynthesis by 40%. At the same concentration, zileuton and Bzlso, inhibited 5-LO products biosynthesis by 98% and 93%, respectively (Figure 4).

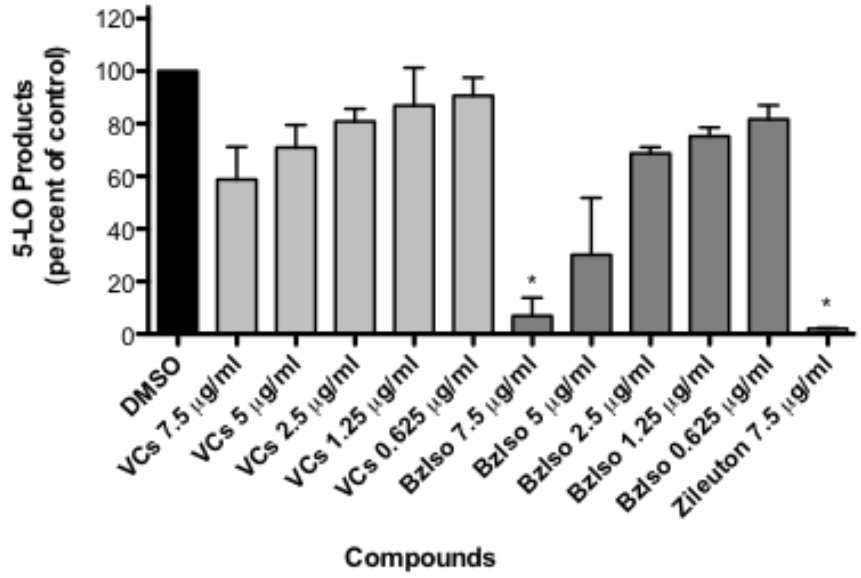


Fig. 4: Effect *T. majus* L. aerial parts volatile components (VCs) and benzyl isothiocyanate (Bzlso) on the biosynthesis of 5-LO products in human neutrophils. Indicated compounds (and 5-LO inhibitor zileuton as control) where pre-incubated with neutrophils for 5 min before cell stimulation. 5-LO products represent the sum of leukotriene B_4, its trans isomers, 20-COOH- and 20-OH-leukotriene B_4 and 5-hydroxyeicosatetraenoic acid.

*Significantly different from control (DMSO), $P<0.05$. Data are expressed as means±SEM of 3 independent experiments.

Compared to Baicalein, no significant effects were observed on the generation of the 12-LO product 12-HETE (Figure 5) by VCs and Bzlso suggesting selective 5-LO inhibition. These data suggest that Bzlso may be a potent and selective 5-LO inhibitor.

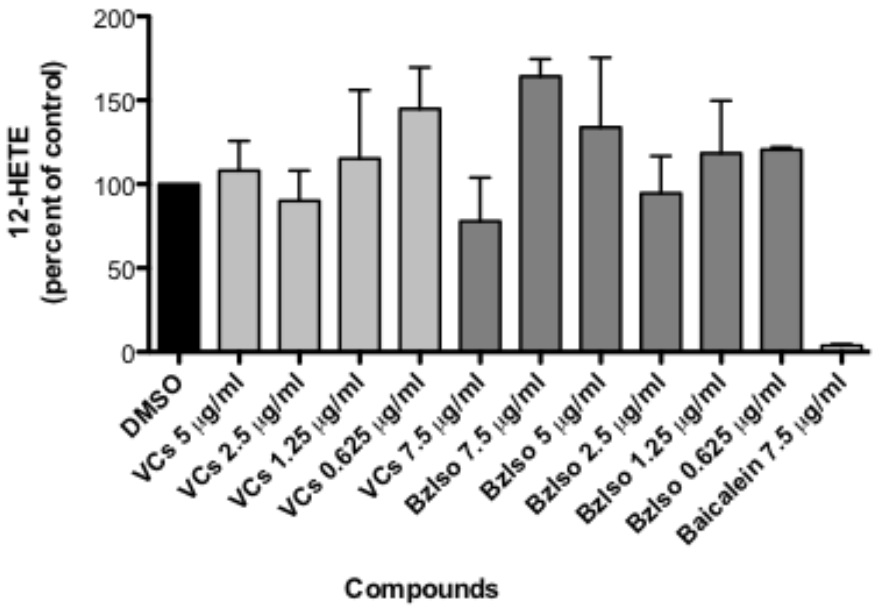


Fig. 5: Effect *T. majus* L. aerial parts volatile components (VCs) and benzyl isothiocyanate (Bzlso) on the biosynthesis of the 12-LO product 12-HETE in human platelets. Indicated compounds (and 12-LO inhibitor Baicalein as control) where pre-incubated with platelets for 5 min before cell stimulation. Data are expressed as means±SEM of 3 independent experiments.

CONCLUSION

Finally, the results show that the volatiles analyzed in our study revealed good antioxidant activity and *lipoxygenase* assay proved that Bzlso is a potential anti-inflammatory compound, so it could be suggested for use as natural antioxidants in food and/or for a new source of nutraceutical foods.

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CONFLICT OF INTEREST STATMENT

The authors declare that there are no conflicts of interest.

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