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Phytochemical profile and antioxidant properties of essential oils isolated from the aerial parts of Rosmarinus officinalis L. cultivated in Northwestern of Tunisia

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The present study aims to study chemical composition and antioxidant capacity of the essential oils isolated from the aerial parts of rosemary (*Rosmarinus officinalis* L.) cultivated in northwestern of Tunisia. Chemical composition of isolated rosemary essential oil was determined by gas chromatography and mass spectrometry (GC-MS). Antioxidant activities were determined *in vitro* using DPPH and ABTS assays. In this research, we identified 31 chemical compounds of the studied rosemary essential oil, and the main constituents were 1,8-cineole (20.07), eucalyptol (18.87), caryophyllene (9.49%), ?-pinene (8.52%), ?-thujone (7.42%) and ledol (6.39). We also showed that *Rosmarinus officinalis* Essential Oil (ROEO) present an important RSA (Radical Scavenging Activity) against DPPH and ABTS but lesser than BHT, used as reference antioxidant molecule. In conclusion we suggest that the proportion of monoterpenes present in the essential oil obtained from *R. officinalis* may be among the active principles responsible for the antioxidant activity shown *in vitro* by ROEO.

Keywords: *Rosmarinus officinalis*, essential oils, phytochemical composition, antioxidant activities

INTRODUCTION

In normal physiological conditions, free radicals are constantly produced in small quantities as tissue mediators or residues of energy or defense reactions. This physiological production is perfectly controlled by endogenous defense systems, which are moreover adaptive in relation to the level of radical presents (Wu et al., 2013). Reactive oxygen species (ROS) are produced by various physiological mechanisms because they are useful for the organism at a reasonable dose, but the production can become excessive and the organism must protect itself through various antioxidant systems (Halliwell and Gutteridge, 2006; Wu et al., 2013).

The imbalance may come from a nutritional and/or antioxidants deficiencies provided by food, such as vitamins or trace elements. However, the intracellular imbalance between the production of free radicals and their degradation induce a state of oxidative stress (Delattre et al., 2005).

ROS (Reactive Oxygen Species) are likely to oxidize surrounding molecules such as nucleic acids, lipids, carbohydrates and proteins causing cell damage leading to necrosis or apoptosis (Bukhari et al., 2016; Michel et al., 2008; Duan and Kasper, 2011; Gonos et al., 2018). These cellular oxidations are also involved in many chronic pathologies such as cancer, diabetes, atherosclerosis, cirrhosis and Alzheimer's disease (Valko et al., 2004; Ridnour et al., 2005).

To protect against this oxidative status, commercial antioxidants such Trolox, butylated hydroxytoluene (BHT) and Butylhydroxyanisol (BHA) are frequently used. These synthetic molecules are usually associated with considerable side effects. Therefore, research has focused on prospecting natural antioxidants found primarily in plants (Jedidi et al., 2019).

Among these, rosemary (Rosmarinus officinalis L., Lamiaceae) is an aromatic and medicinal plant. It is grown worldwide, but it is an evergreen perennial shrub native to southern Europe and Asia, especially the Mediterranean region (Al-Sereiti et al., 1999). In Tunisia, it grows wild in the forests of the North-West. It is also cultivated as an ornamental plant and used in traditional medicine due to its richness in compounds and its economic importance.

However, due to its richness in therapeutically active compounds (Rašković et al., 2014), this plant has many beneficial health effects as antioxidant (Wang et al., 2008), antidiabetic (Sebai et al., 2014), hepatoprotective (Rašković et al., 2014) and anti-inflammatory (Takaki et al., 2008) activities.

The aim of the present study was to examine the chemical composition of the essential oils isolated from the aerial parts of Rosmarinus officinalis by GC/MS and to evaluate their antioxidant capacity.

MATERIALS AND METHODS

Chemicals

Butylated Hydroxytoluene (BHT); 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2'-azino-bis [3-ethylbenzthiazoline-6-sulphonic acid] (ABTS); Diméthylsulfoxyde (DMSO); ethanol; methanol were obtained from Sigma-Aldrich Co.

Plant Material

Rosmarinus officinalis aerial parts were collected in April, 2020 from the Sylvo-Pastoral Institute of Tabarka (North-West of Tunisia) and identified by Dr Imen Bel Hadj Ali, Associate professor in the University of Jendouba-Tunisia. The specimens (No. R101) have been deposited with the herbarium of the Higher Institute of Biotechnology of Béja.

Isolation of essential oils

The extraction of essential oils of aerial parts of rosemary was carried out using steam distillation for 3 h. Briefly, this method consists in separating the fresh plant material (about 20 kg) from the water (15 L) by a grid located a few centimeters from the bottom of the extractor. The heated water and pressure produce steam enriched in volatile constituents. The floral water is collected in separating funnel and the separated essential oil is collected in an opaque glass bottle.

Gas chromatography–mass spectrometry

The essential oils obtained were diluted with n-pentane (Hosni et al., 2008) and analyzed with a Hewlett-Packard 6890N/5975B inert GC-MSD system (Agilent, USA) equipped with two cap. Columns, a HP-INNOWAX (30 m×0.25 mm i.d., film thickness 0.25 µm) and an HP-5MS (30 m×0.25 mm i.d., film thickness 0.25 µm) column (J&W Scientific, USA). The oven temperature was

programmed isothermal at 50°C for 1 min, then rising from 50 to 250 °C at 28/min, and finally held isothermal at 250°C for 15 min; injector temperature, 250 °C; ion source temperature, 230 °C; carrier gas, He (high purity 99.99%; 1.2 ml min-1); injection volume, 1 µl; split ratio, 100:1. The electron impact ionization mode was used with an ionization voltage of 70 eV. Total ion chromatograms were obtained over the scan range of 30-800 a.m.u in the full-scan acquisition mode.

The compounds were identified using the NIST05 and Wiley 7 databases with a resemblance percentage above 85%. Semi-quantitative data were calculated from the GC peak areas without using correction factors and were expressed as a relative percentage (peak area %) of the total volatile constituents identified. Retention indices (RI) were determined for all the detected compounds based on the retention times (tr) of a homologous series of n- alkanes (C8-C30) (Adams, 2007).

Antioxidant Activity

DPPH Radical scavenging activity

The DPPH test was evaluated according to the method previously described by Grzegorczyk et al., (2010). Briefly, various concentrations of essential oils dissolved in dimethyl sulfoxide (10 to 250 µg/mL) were added to 1mL of 0.1 mM DPPH methanol solution and incubated at room temperature in the dark for 30 min. Then the absorbance was measured at 515 nm. Scavenging activity was measured as the decrease in absorbance of the samples relative to the standard DPPH solution. DPPH radical scavenger activity (RSA) expressed as percentage was calculated using the following formula:

$$\%RSA = [(A0 - As)/A0] \times 100;$$

where A0 and As are the absorbance of the control and the sample, respectively. The inhibitory concentration (IC50) expressed in µg ROEO/mL was determined from the graph of free radical scavenging activity (%) versus ROEO concentration. BHT was used as a reference molecule in the same concentration as the test essential oils.

ABTS Scavenging Activity

The antioxidant capacity of ROEO were evaluated using the 2,20-azino-bis [3-ethylbenzthiazoline-6-sulphonic acid] (ABTS) method by following the green color shift by spectrophotometry (Siddhuraju, 2006). The inhibitory concentration 50 (IC50) value was determined as the concentration (µg/mL) of the essential oil required to scavenge 50% of the ABTS radical.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) and were expressed as means ± standard error of the mean (SEM). All samples were analyzed in three replicates. All statistical tests were two-tailed, and results were considered statistically significant when $P < 0.05$.

RESULTS

Chemical composition of ROEO

The essential oil distilled from the Rosmarinus officinalis had a strong odor, and the yield obtained of the essential oil was 1.52 % (v/w in fresh matter). The total number of identified chemical constituents was 31, representing 99.96% of the total oil content. In addition, the percentage of

each compound of ROEO was presented in table 1 and figure 1.

The essential oil contains a complex mixture of monoterpenes and sesquiterpenes. The principal compounds detected are: 1,8-cineole (20.07), eucalyptol (18.87), caryophyllene (9.49%), β-pinene (8.52%), β-thujone (7.42%), ledol (6.39) and bicyclo[2.2.1]heptan-2-one (6.75%).

The obtained results also revealed the presence of other compounds with low proportions such as atis-16-ene (0.97%), 1,4-methanoazulene (0.51%), cis-salvene (0.27), isoborneol (0.20%) and α-cubebene (0.16%).

In vitro antioxidant activity of ROEO

The antioxidant activity of ROEO was evaluated by the DPPH and ABTS free radical scavenging tests and the result was presented by IC50 value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH or ABTS.

The results in table 2 showed that the percentage of inhibition increased in a significant and dose-dependent manner. We also showed that ROEO present an important RSA against DPPH and ABTS (IC50 = 100.6 ± 2.45 and 74.6 ± 1.32 µg/mL, respectively), but lesser than BHT (IC50 = 63.3 ± 1.32 µg/mL) used as reference molecule.

DISCUSSION

In the present investigation, we studied the he phytochemical composition of rosemary essential oil and its antioxidant capacity.

Phytochemical analysis of ROEO allowed identifying 31 compounds. Interestingly, we noticed that the total number of identified components of the present study is significantly higher than those published previously (Sebai et al., 2014; Rašković et al., 2014; Takayama et al., 2016). The main compounds detected in the ROEO are 1,8-cineole (20.07), eucalyptol (18.87), caryophyllene (9.49), β-pinene (8.52), β-thujone (7.42), ledol (6.39) and bicyclo[2.2.1]heptan-2-one (6.75). These variations in the essential oils composition might be related to plant parts, sites and seasons, hence the phenological stage of the plant, and also the essential oil isolation method (Perry et al., 1999; Teixeira et al., 2013). In this respect, phenolic compounds are affected by geographic distribution as well as altitude (Jedidi et al., 2020).

Concerning the in vitro antioxidant activity of ROEO, we demonstrated a relatively high DPPH and ABTS radical scavenging capacity with IC50 values of 100.6 ± 2.45 and 74.6 ± 1.32 µg/mL, respectively. Our results are in line with previously published data. The positive correlation between free radical scavenging activity and chemical composition of essential oils obtained from the rosemary cultivated in Tunisia was previously demonstrated (Kadri et al., 2011). It has also been demonstrated that IC50 values generally vary among studies. This variation can be explained by different chemical compositions of rosemary essential oils.

Importantly, the potent free radical scavenging activity recorded in the present study is due, in part, to the presence of 1,8-cineole, α-pinene and β-pinene (Wang et al., 2008).

On the other hand, the minor components like major compounds may also contribute significantly to the activity of ROEO. This means that oxygenated monoterpenes, probably monoterpenoid ketones with established antioxidant properties, may have the greatest contribution to the antioxidant capacity of ROEO (Viuda-Martos et al., 2010).

In another study, the essential oils from rosemary exerted strong antioxidant activity in vivo and provided restoration of enzymatic and non-enzymatic antioxidant activities, such as superoxide

dismutase, catalse, glutathione peroxidase, sulfhydryl groups and reduced glutathione (Sebai et al., 2014; Selmi et al., 2017).

CONCLUSION

Results of the present study showed that Rosmarinus officinalis essential oils consisted mainly of oxygenated monoterpenes, with 1,8-cineole and eucalyptol as major components. Sesquiterpene hydrocarbons were the least represented compounds. The studied essential oils exhibited higher antioxidant activity, showing a very positive correlation between antioxidant properties and the synergistic effect of compounds in ROEO. Therefore, we suggest that ROEO had potential beneficial effects owing in part to its antioxidant and ROS scavenging activities.

This study of essential oils is helpful in the development of R. officinalis resources for physiological activities, pharmaceutical and fragrance industries. Furthermore, these results can encourage research actions for the exploitation of cultivated strains of R. officinalis that comply with the requirements of different fields of use.

REFERENCES

Adams, R.P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry. In identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream, IL, USA, p. 804.

Al-Sereiti M.R., Abu-Amer K.M., Sen P. (1999). Pharmacology of rosemary (Rosmarinus officinalis L.) and its therapeutic potentials. Indian J. Exp. Biol., 37: 124–130.

Bukhari S.A., Zafar K., Rajoka M.I., Ibrahim Z.R., Javed S., Sadiq R. (2016). Oxidative stress-induced DNA damage and homocysteine accumulation may be involved in ovarian cancer progression in both young and old patients. Turk. J. Med. Sci., 46: 583–589.

Delattre J., Beaudeux J.L., Bonnefont-Rousselot D. (2005). Radicaux libres et stress oxydant (aspects biologiques et pathologiques). 548 p.

Duan J., Kasper D.L., (2011). Oxidative depolymerization of polysaccharides by reactive oxygen/nitrogen species. Glycobiol., 21: 401–409.

Gonos E.S., Kapetanou M., Sereikaite J., Bartosz G., Naparło K., Grzesik M., Sadowska-Bartosz I. (2018). Origin and pathophysiology of protein carbonylation, nitration and chlorination in age-related brain diseases and aging. Aging, (Albany NY), 10: 868–901.

Grzegorczyk I., Matkowski A., Wysokinska H. (2007). Antioxidant activity of extracts from in vitro cultures of Salvia officinalis L. Food Chem.,104: 536–541.

Halliwell B.G., Gutteridge J.M.C. (2006). Free radicals in biology and medicine, Ed 4. Clarendon Press, Oxford, p. 245.

Hosni K., Msaâda K.,Ben Taârit M., Ouchikh O., Kallel M., Marzouk B. (2008). Essential oil composition of Hypericum perfoliatum L. and Hypericum tomentosum L. growing wild in Tunisia. Ind. Crops Prod., 3: 308–314.

Jedidi S., Selmi H., Aloui F., Rtibi K., Jridi M., Abbes C., Sebai H. (2020). Comparative studies of phytochemical screening, HPLCPDAESI-MS/MSLC-HRESIMS analysis, antioxidant capacity and in vitro fermentation of officinal sage (Salvia officinalis L.) cultivated in different biotopes of northwestern Tunisia. Chem. Biod., 17: e1900394.

Jedidi S., Rtibi K., Selmi S., Aloui F., Selmi H., Wannes D., Sammari H., Dhawefi N., Chaâbane A., Sebai H. (2019). Phytochemical/Antioxidant Properties and Individual/Synergistic Actions of Salvia officinalis L. Aqueous Extract and Loperamide on Gastrointestinal Altering Motor Function. J. Med. Food., 22: 1235–1245.

Kadri A., Zarai Z., Ben Chobba I., Bekir A., Gharsallah N., Damak M., Gdoura R. (2011). Chemical constituents and antioxidant properties of Rosmarinus officinalis L. essential oil cultivated from South-Western Tunisia. J. Med. Plants Res., 5: 5999–6004.

Michel F., Bonnefont-Rousselot D., Mas E., Drai J., Thérond P. (2008). Biomarkers of lipid peroxidation: analytical aspects. Ann. Biol. Clin., 66: 605–620.

Perry N.B., Anderson R.E., Brennan N.J., Douglas M.H., Heane A.J., Mc Grimpsey J.A., Smallfield B.M. (1999). Essential oil from Dalmatian sage (Salvia officinalis L.), variations among individuals, plant parts, seasons and sites. J. Agric. Food. Chem., 47: 2048–2054.

Rašković, A., Milanović, I., Pavlović, N. et al. (2014). Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential. BMC. Complement. Altern. Med., 14: 225.

Ridnour L.A., Isenberg J.S., Espey M.G., Thomas D.D., Roberts D.D., Wink D.A. (2005). Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1. Proc. Nat. Acad. Sci. U.S.A., 102: 13147–13152.

Sebai H., Selmi S., Rtibi K., Gharbi N., Sakly M. (2015). Protective Effect of Lavandula stoechas and Rosmarinus officinalis essential oils against reproductive damage and oxidative stress in alloxan-induced diabetic rats. J. Med. Food., 18: 241–249.

Selmi S., Rtibi K., Grami D., Sebai H., Marzouki L. (2017). Rosemary (Rosmarinus officinalis) essential oil components exhibit anti-hyperglycemic, anti-hyperlipidemic and antioxidant effects in experimental diabetes. Pathophysiology, 24: 297–303.

Siddhuraju P. (2006). The antioxidant activity and free radical-scavenging capacity of phenolics of raw and dry heated moth bean (Vigna aconitifolia) (Jacq.) marechal seed extracts. Food Chem., 99:149–157.

Takaki I., Bersani-Amado L.E., Vendruscolo A., Sartoretto S.M., Diniz S.P., Bersani-Amado C.A., Cuman R.K. (2008). Anti-inflammatory and antinociceptive effects of Rosmarinus officinalis L. essential oil in experimental animal models. J. Med. Food., 11: 741–746.

Takayama C., de-Faria F. M., de Almeida A.C.A., Dunder R.J., Manzo L.P., Socca E. A. R., Luiz-Ferreira A. (2016). Chemical composition of Rosmarinus officinalis essential oil and antioxidant action against gastric damage induced by absolute ethanol in the rat. Asian Pacific J. Tropical biomed., 6: 677–681.

Teixeira B., Marques A., Ramos C., Neng N.R., Nogueira J.M.F., Saraiva J.A., Nunes M.L. (2013). Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Ind. Crops Prod., 43: 587–595.

Valko M., Izakovic M., Mazur M., Rhodes C.J., Telser J. (2004). Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cell. Biochem., 266: 37–56.

Viuda-Martos M., Ruiz Navajas Y., Sánchez Zapata E., Fernández-López J., Pérez Álvarez J.A. (2010). Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour Frag. J., 25: 13–19.

Wu J.Q., Kosten T.R., Wang W., Wu N., Zu Y.G., Fu Y.J. (2008). Antioxidative activity of Rosmarinus officinalis L. essential oil compared to its main components. Food Chem., 108: 1019–122.

Zhang X.Y. (2013). Free radicals, antioxidant defense systems, and schizophrenia. Prog. Neuropsychopharmacol. Biol. Psych., 46: 200–206.

References