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Full Length Research Paper

Chemical composition and *in vitro* antioxidant properties of essential oil of *Ricinus communis* L.

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The essential oil from the aerial parts of *Ricinus communis*, an aromatic member of the Euphorbiaceae family, from Tunisia, obtained by hydrodistillation, was analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Seven constituents were found representing 100% of the oil. The main constituents of the essential oil were α-thujone (31.71%), 1,8-cineole (30.98%), followed by α-pinene (16.88%), camphor (12.98%) and camphene (7.48%). Antioxidant activity of the investigated essential oil was evaluated by different test systems: 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, *β*-carotene bleaching test and reducing power assay. The essential oil exhibited a potential antioxidant activity. From the DPPH assay and the *β*-carotene bleaching test, the *R. communis* exhibited a half antioxidant capacity at 300 µg/ml than the positive control, however, the reducing power assay indicate a higher reducing ability of the essential oil. This is the first and original study on chemical composition and antioxidant activity of the essential oil of *R. communis.*

Key words: *Ricinus communis,* essential oil, chemical composition, antioxidant activity.

INTRODUCTION

Ricinus communis is a plant belongs to the family Euphorbiaceae which is a soft-wooded small tree widespread throughout tropics and warm temperature regions of the world (Ivan, 1998). It is an important oilseed crop that produces an oil rich in ricinoleic acid, which confers unique properties to the oil (Velasco et al., 2005; Rojas-Barros et al., 2004; Zhang et al., 2005). This plant grows in Tunisian as a potential source of essential oils, has been used widely as a contraceptive herbal drug in traditional folk medicine. The efficacy of the seeds as a contraceptive drug has been studied with several traditional applications (Devendra and Raghavan, 1978; Gaydou et al., 1982). They have been used with arguable success in the treatment of warts, cold tumors and indurations of the mammary glands, corns and moles (Huguet-Termes, 2001; Gibbs et al., 2002; Wilcox et al., 2004). Its extracts were found to cause proportional increase in mean wheal diameter in skin tests in Castor bean allergic workers (Fakhri, 1989). The anti-inflammatory and the free radical scavenging activity were well demonstrated (Ilavarasan et al., 2006). Nowadays, there is increasing interest in the use of naturally occurring substances for the preservation of food. Plant essential oils and their components have been known to exhibit biological activities, especially

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antimicrobial and antioxidant, and have also been the subject of study, particularly by the chemical, pharmaceutical, and food industries, because of their potential use in food. The reason that antioxidants are important to human physical well being comes from the fact that oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive forms such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radicals, collectively known as active oxygen. These molecules are formed in living cells by various metabolic pathways. Several substances have been proposed to act as antioxidants *in vivo*. Free radicals can also cause lipid peroxidation in foods that leads to their deterioration. Antioxidants play important roles in preventing the diseases induced by reactive oxygen species (ROS) which result in oxidative damage to DNA, proteins and other macromolecules (Fiskin et al., 2006; Ozkan et al., 2007) and are associated with degenerative or pathological events, such as aging (Balaban et al., 2005), asthma (Wilcox and Bodeker, 2004), and cancer (Klaunig and Kamendulis, 2004; Ozkan and Fiskin, 2004). Therefore, research works concerning essential oils as alternative potential antioxidant for treatment of human diseases and for food preservation were important, to prevent the use of synthetic antioxidant such as butylated hydroxy-anisole (BHA) and butylated hydroxytoluene (BHT), which they have been questioned for their safety as carcinogenic. Therefore, research in the determination of natural sources of antioxidants and the antioxidant potential of plants is important. To the best of our knowledge, there has not been elaborate published work on the chemical composition and the antioxidant activity of *R. communis* essential oil. The literature outlines different approaches for determination of the properties of plant extract. Thus, this study was undertaken with the aim to identify the chemical composition of essential oil isolated from the aerial parts of *R. communis* from Tunisia and to evaluate its antioxidant activities by three *in vitro* assay models such as the DPPH free radical scavenging assay, *β*-carotene bleaching test and reducing power assay.

MATERIALS AND METHODS

Chemicals, reagents and plant material

Chemicals and reagents were supported by Prolabo (Paris, France) and Pharmacia (Uppsala, Swedeen). Plant materials (aerial parts) of *R. communis* were collected in the last week of April 2009 from the region of Gargour, Sfax (south of Tunisia).

Distillation of essential oil

The fresh aerial parts of *R. communis* (300 g) were hydrodistilled using a Clevenger-type apparatus to recover the essential oils for 4 h. The distilled essential oils were dried over anhydrous sodium sulfate, filtered and stored at +4°C.

GC/MS analysis conditions

The essential oil was analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 250 and 280°C, respectively. The column temperature was programmed from 35 to 250°C at a rate of 5°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. The flow rate of the carrier gas (helium) was 1.0 ml/min. A sample of 1.0 µl was injected, using split mode (split ratio, 1:100). All quantifications were carried out using a built-in data-handling programme provided by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituents was based on a comparison of their retention times to *n*-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their mass spectra compared to the Wiley version 7.0 library.

Antioxidant activity tests

DPPH radical scavenging assa*y*

The ability of *R. communis* oil to scavenge free radicals was assayed with use of a synthetic free radical compound, 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method employed by Bersuder et al. (1998). Briefly, a volume of 500 µl of each sample was mixed with 500 µl of ethanol and 125 µl (0.02%, w/v) of DPPH in 99.5% ethanol.

The mixture was shaken vigorously and incubated in the dark. After 60 min, the absorbance was measured at 517 nm using a spectrophotometer. The DPPH radical-scavenging activity was calculated as follows:

$$\text{Radical-scavenging activity} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100,$$

Where A_{blank} and A_{sample} are the absorbance of the control (blank) and the sample, respectively. The IC_{50} value was defined as the amount of antioxidant necessary to inhibit DPPH radical formation by 50%. The synthetic antioxidant reagent BHT was used as a positive control.

The values are presented as the means of triplicate analysis. β-carotene bleaching assay. The antioxidant assay using the β-carotene bleaching was determined according to the protocol previously described (Koleva et al., 2002). β-Carotene (0.5 mg) was dissolved in 1 ml of chloroform and mixed with 25 µl of linoleic acid and 200 µl of tween 40. The chloroform was evaporated under vacuum at 40°C, then, 100 ml of distilled water was added and the resulting mixture was vigorously stirred. About 2.5 ml of the obtained emulsion was transferred into different tubes containing 500 µl of essential oil dissolved in absolute ethanol at different final concentrations (5 to 70 µg/ml). The tubes were immediately incubated at 50°C for 120 min and the absorbance was measured at 470 nm before and after heat treatment. A control blank containing 0.5 ml of ethanol instead of the sample test was carried out in parallel. Synthetic antioxidant butylated hydroxytoluene (BHT) was used as positive control and all tests were carried out in triplicate.

Table 1. Chemical composition of *R. communis* essential oil.

No.	Compound	RI	%	Identification
2	α- pinene	932	16.88	MS, RI
3	camphene	948	07.48	MS, RI
4	1,8-cineole	1044	30.98	MS, RI
5	α-thujone	1131	31.71	MS, RI
6	camphor	1174	12.92	MS, RI

The components and their percentages are listed in order of their elution on apolar column (HP-5), RI: Retention indice, %: Percentage.

Table 2a. Antioxidant activity of R. communis essential oil and positive control (BHT) with the free radical DPPH scavenging assay.

Sample	DPPH (%)			IC_{50}[a]
	100[a]	200[a]	300[a]	
Oil	19.37±0.30	27.30±2.30	46.30±2.43	307.00
BHT	80.00±1.50	80.00±2.10	80.70±2.40	40.50

[a]Concentration (µg/ml). Values represent average of triplicates ± standard deviation.

Reducing power antioxidant

The ability of oil to reduce iron (III) was determined according to the Yildirim's method (Yildirim et al., 2001) with some modifications. An aliquot of 500 µl of each sample at different final concentrations was dissolved in ethanol and mixed with 1.25 ml of reagent of 0.2 M phosphate buffer (pH 6.6) and 1.25 ml of 1% potassium ferracyanide.

The mixture was incubated 30 min at 50°C, followed by addition of 1.25 ml of 10% (w/v) trichloroacetic acid. The mixture was then centrifuged at 1500 g for 10 min. Finally, 1.25 ml of the supernatant solution was mixed with 1.25 ml of distilled water and 250 µl of 0.1% (w/v) ferric chloride. After 10 min, the absorbance was measured at 700 nm spectrophotometerically. Increased absorbance of the reaction mixture indicated increased reducing power.

Synthetic antioxidant BHT was used as positive control and all tests were carried out in triplicate.

RESULTS AND DISCUSSION

Chemical composition

The results obtained by GC-MS analyses of *R. communis* essential oil using capillary columns are presented in (Table 1.) The constituents were identified and their percentages listed according to their elution order on the HP-5MS column. A total of seven compounds were identified, accounting for 99.97% of the oil with a yield of 0.32%. As can be seen from (Table 1), the oil was characterized by a high amount of monoterpenes including 24.36% of monoterpene hydrocarbons and 75.61% of oxygenated monoterpene. The main constituents were α-thujone (31.71%) and 1,8-cineole (30.98%). Additionally, in a considerable amount, α-pinene (16.88%), camphor (12.98%) and camphene (7.48%) were also detected (Table 1). Our literature survey could not find a report on the chemical composition of *R. communis* essential oil from others countries.

Antioxidant activity

It is known that free radicals are involved in the process of lipid per-oxidation and play a cardinal role in numerous chronic diseases such as cancer and coronary heart disease (Halliwell and Gutteridge, 1999). Thus, the ability to scavenge free radicals is an important antioxidant property in order to minimize oxidative cellular damage. For, this, various *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts and essential oil. These methods are popular due to their high speed and sensitivity. However, it is crucial to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals (Salazar et al., 2008). In this study, three complementary test systems, namely DPPH free radicals scavenging, *β*-carotene bleaching test and reducing power assay were assessed.

DPPH radical-scavenging activity

The antioxidant activity of the volatile compounds from *R. communis* essential oil was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. The method is based on the reduction of alcoholic $DPPH^{\cdot}$ solutions in the presence of a hydrogen donating antioxidant. $DPPH^{\cdot}$ solutions show a strong absorption band at 517 nm appearing as a deep violet color. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining $DPPH^{\cdot}$, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant. In the present study, the results of the free radical scavenging activity of *R. communis* assessed by DPPH assay was summarized in (Table 2a). The essential oil exhibited a dose dependent increase with lower DPPH radical scavenging abilities of 19.37 ± 0.30, 27.30 ± 2.30 and 46.30 ± 2.43% at 100, 200 and 300 µg/ml, respectively. Comparison of these results with those expressed by BHT showed that *R. communis* exhibited a moderate antioxidant effect than BHT (46.30 ± 2.43 *vs*. 80.70 ± 2.40% at 300 µg/ml) which

Table 2b. Antioxidant activity of R. communis essential oil and positive control (BHT) with β-Carotene bleaching method.

Sample	β-Carotene linoleic acid (%)			IC_{50}[a]
	10[a]	40[a]	70[a]	
Oil	12.34±1.80	33.50±3.00	39.47±2.00	-
BHT	27.00±3.00	70.30±2.00	77.50±1.00	20

[a]Concentration (µg/ml). Values represent average of triplicates ± standard deviation.

Table 2c. Antioxidant activity of R. communis essential oil and positive control (BHT) with the reducing power test.

Sample	Reducing power (absorbance at 700 nm)			EC_{50}[a]
	10[a]	40[a]	70[a]	
Oil	0.151±0.025	0.512±0.022	0.804±0.013	39.32
BHT	0.390±0.024	0.891±0.024	1.051±0.012	13.80

[a]Concentration (µg/ml). Values represent average of triplicates ± standard deviation.

is about the half. The amount of the essential oil needed for 50% inhibition of free radical activity is expressed by IC_{50}. Lower IC_{50} value indicates higher antioxidant activity. As shown from (Table 2a), the essential oil activity was found to be 7 to 8 times lower than that of synthetic antioxidant BHT. The antioxidant activity found is attributed to the number of hydroxyl groups that are available to donate hydrogen atoms to the DPPH radical.

As shown in (Table 1), the *R. communis* essential oil is poor in oxygenated sesquiterpenes, which may act as a reducing factor of the antioxidant activity. The weaker DDPH Scavenging effect was attributed to the presence of oxygenated monoterpenes (75.61%). This result was confirmed by Ruberto and Baratta (2000).

β-Carotene bleaching method

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. Hence, the free radical linoleic acid attacks the highly unsaturated *β*-carotene, and the presence of different antioxidants can hinder the extent of *β*-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system. There was absorbance decreased rapidly in samples without antioxidant, whereas in the presence of an antioxidant the colour was retained for a long time. (Table 2b), shows the antioxidant activity of the essential oil and BHT as measured by the bleaching of the *β*-carotene–linoleate system. At 70 µg/ml, the oil showed lower linoleic acid inhibition activity than BHT, which is almost half to the synthetic antioxidant BHA (39.47 ± 2.00% *vs.* 77.50 ± 1.00% for essential oil and BHT, respectively). Such weak antioxidant activity was related to the dominance of non phenolic compounds in *R. communis* essential oil.

Reducing power antioxidant

Reducing power assay measures the electron-donating capacity of an antioxidant. The reducing properties are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation (Matsushige et al., 1996). Being good electron donors, phenolic compounds show the reducing power and have ability to convert the ferric ion Fe^{3+} to ferrous ion Fe^{2+} by donating an electron (Shon et al., 2004). Increasing absorbance at 700 nm indicates an increase in reductive ability. (Table 2c) shows dose-response curves for the reducing powers of essential oil. It was found that the reducing power (absorbance at 700 nm) value at the final concentration (70 µg/ml) were 0.804 ± 0.010, which is slightly lower than that of BHT (1.051 ± 0.010). The EC_{50} value of *R. communis* essential oil was 39.32 µg/ml which was about three times lower than BHT (13.80 µg/ml).

The ability to reduce Fe(III) may be attributed to hydrogen donation from phenolic compounds (Shimada et al., 1992), which is also related to the presence of reductant agent. The higher reducing power of essential oil may be attributed to the presence appreciable amount of 1,8-cineole, α-pinene and camphene.

Conclusion

In recent decades, essential oil of plants have drawn great importance as sources of natural products in both academia and the food, cosmetic and pharmaceutical industries, since their possible use as natural additives emerged from a growing tendency to replace synthetic preservatives by natural ones. The results reported here can be considered as the first information on the chemical composition and the antioxidant activity of the essential oil of *R. communis*. We can conclude that the overall results obtained by reducing power and β-Carotene bleaching test were better than those provided by the radical- scavenging activity. The difference is probably as a consequence of a higher specificity of the assay for lipophilic compounds and the presence of

reductant agent. Nevertheless, further studies need to be carried out to define the active principle(s) of fractions and to study the relation between chemical structure and antioxidant activity *in vitro* and *in vivo* to clarify their biological properties.

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