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CHEMICAL COMPOSITION AND DNA DAMAGE PROTECTIVE EFFECT OF ESSENTIAL OIL OF ROSMARINUS OFFICINALIS AND POPULUS ALBA

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ABSTRACT

There are many plants which were the subject of recent research in the fields pharmaceutical, cosmetic and agroalimentary because of their chemical composition and their several therapeutic activities. Among these plants, the species of *Rosmarinus officinalis* (rosemary) and *Populus alba* (white poplar).The present study aims to determine the chemical composition of the essential oils isolated from these plants by Gas Chromatography/Mass Spectrometry. Then, the study of the efficiency of essential oil to protect the DNA against the free radicals damages was performed. The results of GC/MS analysis reveal that the major components determined in *Rosmarinus officinalis* essential oil were Camphor (22.35%), Verbenone (23.48%), Borneol (16.63%), and Eucalyptol (11.73%) while the major components determined in *Populus alba* essential oil were 1,8-Cineole (38.02%), β-Eudesmol (20.58%) and δ-Cadinene (8.30%). The essential oil of *R. officinalis* and *P. alba* also presented protection against DNA scission induced by OH^- radicals generated from photolysis UV / H_2O_2.

Key words: *Rosmarinus officinalis*, *Populus alba*, Essential oils, GC/MS, DNA.

INTRODUCTION

Rosmarinus officinalis L. (Rosemary) belongs to the family *Labiatae* or *Lamiaceae*. It occurs as a shrub, under shrub or herbaceous (Atik bekkara *et al.,* 2007). It is a dense aromatic plant with dark green lavender like leaves, is a native of the Mediterranean region. In traditional medicine, Rosemary is used to treat different diseases including: depression, insomniac and arthritic pains (Zargari, 1995). The flowering tops and the rosemary leaves mainly contain flavonoids, phenolic acids-especially rosmarinic acid (choleretic activities), and an essential oil (containing pinene, camphene, cineole, borneol and camphor) to which it must have stimulatory effects (Oluwatuyi *et al.*, 2004). Rosemary oil has been widely used for centuries as an ingredient in cosmetics, soaps, perfumes, deodorants, both for flavoring and preservation of food products (Arnold *et al.,* 1997). The Rosemary oil has also many therapeutic and they help the distribution of drugs and antiseptics (Palevitch and Yaniv, 1991). The chemical composition of rosemary oil from different countries has been a subject of extensive study (Fournier *et al.,* 1989), (Chalchat *et al.*, 1993), (Abdelaziz *et al.,* 2000), (Diab *et al.,* 2002), (Mauhssen and Rached, 2003).

Populus alba (White Poplar) is a woody angiosperm higher plant belonging to the family *Salicaceae.* It is a common species of the Mediterranean forests. According to several Mediterranean floras, this species is considered as cultivated or sub-spontaneous around the western Mediterranean Basin **(**Dickmann and Kuzovkina, 2008). White poplar wood is a very poor fuel, which produces little heat to combustion. From the bark, we extract salicin **(**Jean-Claude *et al.,* 2008). The exudate from buds of many species of the Populus genus has long been widely used in medicine for treating wounds and ulcers.

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The antiseptic properties of poplar buds exudate and are mainly due to phenol carboxylic acids (PCA) and flavonoids contained in them. The same components were principally used to determine the poplar resistance to microbial infection (Scaysbrook *et al.,* 1992).

Keeping in view the properties of rosemary and white poplar, the present study aims to determine the chemical composition of the essential oils isolated from these plants by Gas Chromatography/Mass Spectrometry. Then, we have to study the efficiency of essential oil to protect the DNA against the free radicals damages.

MATERIALS AND METHODS

Plant material and essential oil extraction:

It was constituted of aerial parts (leaves and flowers) of the two plant species; *Rosmarinus officinalis* and *Populus alba*. The plants were collected in the Mascara region. They were identified by the botanist of the department of Biology. The extraction of essential oils (EO) from two plants (*Rosmarinus officinalis* and *Populus alba*) was conducted in the laboratories of the University of Mascara. The extraction of essential oils was carried out by hydrodistillation in a Clevenger apparatus. 100 g of leaves and flowers of each plant was boiled. When the temperature stabilizes, the distillate was collected. 18 g of sodium chloride (NaCl) was added to the distillate. Then, the mixture was placed in a separating funnel and three successive washes (10, 10, 20 ml) of cyclohexane were achieved. After agitation, the organic phase was recovered. The concentration of organic phase was achieved by rotary evaporator to obtain the essential oil. The essential oil obtained was stored at + 4 ° C after the calculation of the yield of extraction.

Gas chromatography/mass spectrometry analysis:

The identification of different chemical compounds was realized by gas phase chromatography (TRACE GC-ULTRA, S/N 20062969, Thermo-Fischer) coupled with mass spectrometry (Polaris Q, S/N 210729, Thermo Fischer) (GC/MS). The essential oil was also analyzed by an Agilent-Technologies GC–MS consisted of a 6890A GC system coupled with a 5975C network mass selective detector. Separation of the essential oil chemical constituents was carried out equipped with HP-5 MS capillary fused silica column (30 m x 0.25 mm, 0.25 µm film thickness). The 5%-Phenyl-methylpolysiloxane was used for the analysis of the essential oil. The column temperature was programmed to be heated from 60 °C to 240 °C at a rate of 4°C/min. The helium was used as carrier gas at a flow rate of 1.5 mL min^{-1}. The temperature of the injector was fixed to 250°C. A 1 µL sample volume was injected into the column using the split mode (split ratio 1:100). GC/MS detection was performed by an electron ionization system, with ionization energy of 70 eV and scanned mass range was set at 50–550 m/z. The essential oil components were identified on the basis of comparison of their mass spectra, retention times and retention indices with those of mass spectral library.

DNA damage protective effect of essential oil:

The pharmacological properties of medicinal plants have been widely studied and it was reported to show the different antioxidant activities (Aslam *et al.*, 2011). So, our study was designed to assess the ability of essential oils of *R. officinalis* and *P. alba* to inhibit the DNA damage induced by free radicals.

The induction of DNA cleavage by Fenton's reagent was measured on plasmid pBR322 DNA according to the procedure described by Russo *et al.*, (2000) and Lee *et al.*, (2002). The experiment was done by adding a volume of 20 µl containing 33 µM in pb of the plasmid pBR322 DNA in a saline 5 mM phosphate buffer (pH 7.4). This mixture was added to different concentrations of the essential oil of *Rosmarinus officinalis* and *Populus alba* (25 µg / ml, 50 µg / ml, 100 µg / ml and 200 µg / ml).

Immediately prior to irradiation of the samples with UV light, hydrogen peroxide (H_2O_2) was added to a final concentration of 2.5 mM. The reaction volumes were organized in the caps of centrifuge tubes polyethylene, placed directly on the surface of a transilluminator (8000 $\mu W.Cm^{-1}$) at 300 nm. The samples were irradiated for 5 min at room temperature.

After irradiation, 4.5 µl of a mixture containing 0.25% bromophenol blue, 0.25% xylene cyanol FF and 30% glycerol were added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% horizontal Agarose gel in Tris borate buffer (45 mM Tris-borate, 1 mM EDTA). The untreated pBR322 plasmid was included as a control in each series of electrophoresis gel, conducted at 1.5 V / cm for 15 h. Then, the gel was stained with Ethidium bromide (1 µg / ml, 30 min) followed by a discoloration in water at 30 min. Then, the gel was photographed on a positive film type Polaroid-667.

RESULTS AND DISCUSSIONS

Extraction yields:

The essential oil content, obtained from the aerial parts (leaves + flowers) is 1.29% for the Rosemary and 0.9% for white poplar (Table 1). The yields of essential oils from two species are widely variable.

The yield of essential oil of *R. officinalis* is higher than that quoted by Atik Bekkara *et al.*, (2007) and those of Rouabeh (2010) where the quantities obtained by these two works were respectively 0.8% and 0.9%. Indeed, the extraction yield, as the quality of EO, was influenced by the type of soil on which the planting was done, the material of the equipment used, the cleanliness of the equipment, the operating pressure, regularity the

heating, the cooling of the distillate, method and distillation time (Brulé and Pecout, 1995).

Chemical composition of essential oil:

The chemical components determined in the EO were given in Table 2. Twenty one components in the EO of *R. officinalis* were identified. The major constituents (> 5%) in the EO were found to be Camphor (22.35%), Verbenone (23.48%), Borneol (16.63%), and Eucalyptol (11.73%), respectively. In addition, the tested EO also contained considerable amounts of various minor constituents (Table 2).

There were many studies in the literature on the chemical composition of *R. officinalis* essential oil (Celiktas *et al.,* 2007), (Bousbia *et al.,* 2008). The chromatogram of EO of *R. officinalis* showed a spectrum with high percentage on Verbenone belongs to sesquiterpenes group with high levels of camphor, eucalyptol and Borneol in monoterpenes group. Little variation in the chemical compositions of *R. officinalis* EO across countries might be due to different ecological conditions (Benhabiles and Aït Ammar, 2001), (Lograda *et al.,* 2014). Our results were in agreement with the findings of Miladi *et al.,* (2013) and Kukerja *et al.,* (2007). They also identified Camphor as a major component of *R. officinalis* essential oil. Ayadi *et al.,* (2011) identified Verbenone as a major component of *R. officinalis* essential oil. While chromatographic GC / MS analysis of the EO of *P. alba* has allowed us to identify Twenty two compounds. The main constituents (> 5%) in the EO of *P. alba* were: 1,8-Cineole (38.02%), β-Eudesmol (20.58%), δ-Cadinene (8.30%), α-Eudesmol (6.75%). In addition, the EO of *P. alba* also contained minor amounts of various components (Table 3). An immediate observation on the chromatogram of the EO of *P. alba* focused on the presence of high levels of 1,8-Cineole, Eudesmol and δ-Cadinene that belong to the group of sesquiterpenes. Sesquiterpenes compounds were minor components in vegetable oils. They often give it the essential characteristics of the flavor (Banthrope, 1996).

The α-Eudesmol was differentiated from β-Eudesmol not only by the retention indices, but also by the mass spectra (Adams, 1995). The Eudesmols derivatives were obtained by cyclization of the hedycaryol during aging of the plant or during the extraction process of the EO by distillation (Cornwell *et al.,* 2000). The high percentage of Eudesmols oil could be of interest according to the work of Miyazawa *et al.,* (1996) who studied the antimutagenic activity of essential oil of *Dioscorea japonica.* The small fraction of monoterpenes was dominated by Linalool (0.46%), β-Cyclocitral (0.46%) and Methyl Eugenol (0.52%). The GC / MS analysis of the EO of *P. alba* showed the absence of volatile Aglycone (salicylic alcohol). However, we identified salicylic aldehyde (0.11%). This compound may be derived from salicin and / or Populin by hydrolysis and oxidation.

The variations found in the chemical composition on the qualitative and quantitative point of view of our samples compared to some previous studies may be due to some environmental factors, the part of the plant used, the age of the plant and the period of the growing season or even to genetic factors (Hussain, 2009), (Anwar *et al.,* 2009). The methods that used water can induce hydrolysis of esters and also rearrangements, isomerizations, racemizations, oxidations (Bruneton, 1993).

DNA damage protective effect of essential oil:

Figure 01 represented the electrophoretic profile of DNA of plasmid pBR322 after oxidation with hydrogen peroxide (H_2O_2) 2.5 mM / UV photolysis in the absence and presence of different concentrations of EO of *R. officinalis* and *P. alba* (25, 50, 100 and 200 µg / ml).

The plasmid pBR322 DNA showed two bands by electrophoresis on Agarose gel. The band moves faster was the native form of supercoiled DNA and the slowest group was open circular form. UV radiation of the DNA in the presence of H_2O_2 has allowed the cleavage of supercoiled DNA in linear form and open linear form. The addition of the EO of *R. officinalis* (Line 5-8) and the EO of *P. alba* (line 9-12) to the mixture presented a slight reduction in the formation of the linear DNA (Figure 1). This reduction was recorded in the line 8 which carried the concentration of 200 µg / ml of the EO of *R. officinalis.* Natural compounds from EO of *R. officinalis* and *P. alba* can influence on the oxidation of DNA by a simple mechanism. It was explained by the quenching of oxygen radicals by giving hydrogen or electrons leading to inhibition of the Fenton reaction. So, it could also trap directly OH^- and the protection of the supercoiled plasmid DNA (Prakash *et al.,* 2007). It has been described recently that borneol, as essential oil component of rosemary, was able to protect cells against DNA damage induced by H_2O_2 in rats (Slamenova *et al.,* 2008), (Horváthová *et al.,* 2009). Slamenova *et al.,* (2002) reported that long-term preincubation (24 h) V79 hamster cells with 30 µg / ml of rosemary caused a significant decrease in DNA damage.

Figure 1: Essential oil protective effect of *R. officinalis* and *P. alba* against oxidation of the plasmid pBR322 DNA with hydrogen peroxide / UV

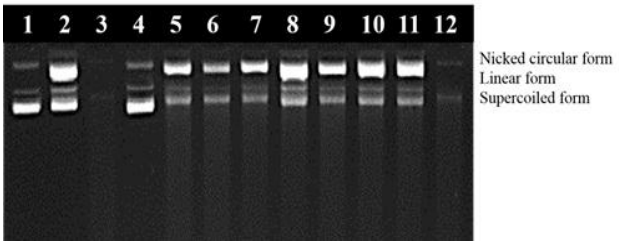

Table 1. Extraction yields

Plants	Mass of plant (g)	Mass of extract (g)	Aspect	Color	Y (%)
R. officinalis	850	11	oily	yellow	1,29
P. alba	850	7,8	oily	pale yellow	0,9

Table 2. Chemical composition of rosemary essential oil

Peak	Constituents	*RT (min)	**RI	Percentage (%)
1	1-Octen-3-ol	13,783	972	0,20
2	Eucalyptol	15,904	1036	11,73
3	Linalool	18,083	1097	6,38
4	Chrysanthenone	18,373	1106	0,32
5	Chrysanthenone	19,163	1130	0,35
6	Camphor	20,131	1158	22,35
7	Borneol	20,85	1178	16,63
8	Isopinocamphone	21,104	1185	4,84
9	α-Terpinol	21,571	1197	3,32
10	Myrtenol	21,881	1206	0,72
11	3-Cyclopentene-1-ethanol, 2,2,4-trimethyl-	22,05	1212	1,67
12	Verbenone	22,479	1225	23,48
13	Grandlure II	23,193	1246	1,33
14	5-Caranol	23,435	1254	2,10
15	p-Mentha-1,8-dien-3-one	24,317	1279	0,35
16	Borneol	24,723	1290	1,36
17	3,5-Heptadienal, 2-ethylidene-6-methyl	28,098	1393	0,39
18	Methyleugenol	28,34	1401	0,41
19	Caryophyllene oxide	34,41	1602	0,94
20	Sabinene	35,848	1654	0,63
21	Caryophyllene oxide	36,385	1673	0,49

*RT: Retention time obtained by chromatogram.
**RI: Retention indices were determined by GC-MS.

Table 3. Chemical composition of White poplar essential oil

Peak	Constituents	*RT (min)	**RI	Percentage (%)
1	δ-Cadinol	4,192	1030	3,09
2	Methyl eugenol	4,592	1185	0,52
3	α-Eudesmol	4,830	1292	6,75
4	Linalool	6,058	1478	0,46
5	Docosane	6,282	1519	0,68
6	3-Menthyl-2-phenylethyl butanoic acid	6,717	1575	0,39
7	3-Phenyl-2-propen-1-ol	6,847	1585	1,15
8	(E)-2-Methyl-2-buten-1-ol	7,237	1616	3,49
9	1,8-Cineole	7,467	1633	38,02
10	α-Copaene	7,723	1697	0,82
11	β-Cyclocitral	8,699	1743	0,46
12	β-Caryophyllene	8,813	1792	0,11
13	β-Eudesmol	9,107	1918	20,58
14	Alloaromadendrene	11,808	1934	0,81
15	δ-Cadinene	12,477	1982	8,30
16	α-Muurolene	12,559	1999	2,73
17	α-Copaene-11-ol	13,971	2156	5,77
18	1-Phenyl-2-propen-1-ol	14,069	2189	0,23
19	Decanoic acid	14,112	2230	0,1
20	(E)-2-Methyl-2-butenoic acid	14,529	2235	0,78

21	Salicyl aldehyde	14,613	2252	0,11
22	p-Cymen-8-ol	14,782	2391	4,64

CONCLUSION

In this study, we performed the extraction of *R. officinalis* and *P. alba* essential oil. The extract was subjected to preliminary phytochemical screening by GC/MS for the detection of natural compounds of these essential oils. Then, the protective effect of each essential oil against the DNA damages was studied. The result of phytochemical screening of essential oils revealed the presence of Camphor (22.35%), Verbenone (23.48%), Borneol (16.63%) and Eucalyptol (11.73%) as major compounds in *R. officinalis* essential oil while the major compounds in *P. alba* essential oil were the1,8-Cineole (38.02%), β-Eudesmol (20.58%), δ-Cadinene (8.30%), α-Eudesmol (6.75%). In another hand, the results of electrophoresis on Agarose gel allowed us that the essential oil of *R. officinalis* and *P. alba* presented a slight reduction in the formation of the linear DNA.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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