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Antioxidant effect of oregano (*Lippia berlandieri* v. Shauer) essential oil and mother liquors

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Abstract

The conventional steam distillation process for oregano (*Lippia berlandieri* v. Shauer) essential oil extraction produces large volumes of mother liquor. This residual liquid represents a potential value because the soluble antioxidants it contains. Essential oil and ethyl acetate mother liquor extracts (MLEs) were evaluated for antioxidant activity. Total phenolic content and antioxidant activities by the 2-2′-diphenyl-1-picrylhydrazyl (DPPH) method, by the deoxyribose degradation assay, and by oxidation of low density lipoproteins (LDL) with $CuSO_4$ were evaluated. Oil yield was 4.34%. Total phenolic content was 151 ± 2.00 and 150.5 ± 0.98 mg of GAE (gallic acid equivalents)/mL for the essential oil and MLEs, respectively. DPPH assay showed a low radical scavenging activity (RSA) for oregano essential oil. Meanwhile MLEs exhibited no significant RSA at low concentrations, but at higher concentrations (100 μg/mL), it was superior to those exhibited by the controls ascorbic acid and butylated hydroxytoluene (BHT). Deoxy-D-ribose assay results for both essential oil and MLEs showed a good hydroxyl radical RSA at the concentrations tested. Essential oil and MLEs delayed induction time effectively. Solubility problems, chemical constituents, and their hydrophilic–lipophilic distribution are key factors that explain samples behavior for an eventual use of these natural products.

Keywords: Antioxidant; LDL oxidation; Mother liquor extracts; Oregano essential oil

1. Introduction

Biological systems are rich in lipids that are prone to oxidation unless they are protected by endogenous or exogenous factors known as antioxidants. These antioxidant compounds can be classified according to their mechanism of action (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003).

The chelating action of phenols over transition metals has already been described already by Brown, Khodr, Hider, and Rice-Evans (1998). Also the radicals scavenging capacity of polyphenols in polar phase (Rice-Evans, Miller, & Paganga, 1996) and lipidic conditions (Sawa, Nakao, Akaike, Ono, & Maeda, 1999) has been reported.

Polyphenols assist the regeneration of tocopherols to their active form, inhibiting several types of oxidative

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enzymes. The diversity of action mechanisms turns polyphenols into an attractive natural source of antioxidants and prophylactic promoters. Although numerous techniques are available to evaluate antioxidant activity, there is no single procedure capable of integrating the full set of mechanisms typical of an antioxidant (Frankel & Meyer, 2000). Thus, to evaluate a given sample, a variety of tests have to be implemented. These antioxidant assays can be categorized into two groups: (a) assays for radical scavenging activity and (b) assays for measuring lipid oxidation inhibition (Puertas-Mejía, Hillebrand, Stashenko, & Winterhalter, 2002).

Traditionally oregano has been consumed as a spice. However, recently there is more interest in other potential uses, particularly as an essential oil, which has proven as a good antimicrobial and antioxidant agent. Most studies have been performed on European oregano (*Origanum vulgare* L.), on the essential oil (Kulisic, Radonic, Katalinic, & Milos, 2004) and volatile (Milos, Mastelic, & Jerkovic, 2000) and non-volatile extracts (Vekiari, Oreopolou, Tzia, & Thomopoulos, 1993). Related reports on *O. vulgare* (Pizzale, Bortolomeazzi, Vichi, Uberegger, & Conte, 2002) have associated phenolic compound content with antioxidant activity. In the case of *Lippia berlandieri* v. Shauer, however there is little documented so far. Independent of oregano species, the steam distillation process for producing the essential oil generates large volumes of mother liquor. Besides the waste disposal problem, this liquor represents an additional value because of its water soluble phenolics. To the best of our knowledge, these have not yet been fully analyzed. The aim of this work was to evaluate the antioxidant effect of the essential oil and mother liquor from oregano (*L. berlandieri* v. Shauer).

2. Materials and methods

Wild dry leaves from *L. berlandieri* harvested at José Maria Morelos y Pavón Ejido in Durango, Mexico, were selected for steam distillation according to the standard procedure (Dorman et al., 2003).

Phenol content in essential oil and mother liquor extracts (MLEs) was determined following the method of Singleton, Orthofer, and Lamuela-Raventos (1999). MLEs were obtained previously by extraction with ethyl acetate following the method proposed by Oszmianski and Sapis (1989).

Free radical scavenging activity (RSA) was measured as the ability of samples to neutralize the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) following the method proposed by Gyamfi, Yonamine, and Aniya (2000). Additionally, the hydroxyl radical scavenging was evaluated using the deoxyribose degradation assay described by Re et al. (1998).

Antioxidant activity of essential oil and MLEs in the induced oxidation of low density lipoproteins (LDL) by $CuSO_4$ was evaluated according to the modified method described by Esterbauer, Gebicki, Puhl, and Jürgens (1992). The response in an accelerated oxidation assay performed using an oxidative stability instrument (OSI), using soy oil without antioxidants as a reference was evaluated. From the data the induction onset was calculated and taken as a parameter, which is the time before an accelerated oxidation change occurs, i.e., a measure of time before manifestation of oxidation. This point was determined graphically as shown in Fig. 1. For this test, protein content was evaluated by Bradford (1976) method.

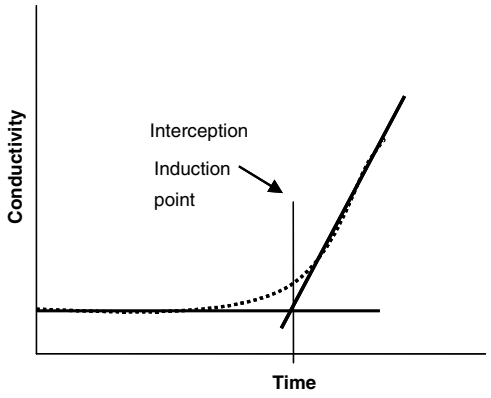

Fig. 1. Graphical method for determination of the induction time onset.

All experiments were performed in duplicate and data analyzed using one way ANOVA analysis with significance level of $p \leqslant 0.05$. Standard deviation of results was obtained using Statistical Software (StatSoft, Tulsa, OK) module Basic Statistics v. 5.2.

3. Results and discussion

The yield of oregano essential oil was 4.34% (w/v), which is slightly higher than that reported previously (Kulisic et al., 2004). The oil content in oregano is around 0.1–3.0%, but in some cases yields can be higher depending on the plant type, phenology and morphology. Meanwhile, the MLEs yield was 0.35% (w/v).

Total phenolic content was 151 ± 2.00 and 150.5 ± 0.98 mg GAE (gallic acid equivalents)/mL for the essential oil and MLEs, respectively. There were no significant statistical differences in phenolic content for both sources ($p = 0.664$). Results obtained are interesting, because diverse plants have been highly correlated with their antioxidant capacity as a function of their total phenolic content (Andarwulan, Fardiaz, Wattimena, & Shetty, 1999), then both essential oil and MLEs derived from the oregano oil extraction might show equivalent antioxidant capacity. However, many authors have reported lack of such a correlation in different natural sources, including plant extracts such as oregano (Dorman et al., 2003). It has been shown that having the same level of phenolic content does not necessarily mean the same antioxidant response (Parejo et al., 2002). It is known that antioxidant effect and its evaluation depends on factors such as the hydrophilic–lipophilic balance of the antioxidant extract, the chemistry of the surrounding medium (Chang et al.,

2003), the analytical assays used, and the chemical properties and structure of active compounds.

Results from DPPH assay show that oregano essential oil at 1 and 5 μg/mL exhibits a radical scavenging activity of about 6%, which is low but slightly superior to that of butylated hydroxytoluene (BHT) and ascorbic acid used as controls at the same concentrations (see Figs. 2 and 3). Moreover, no increase in the RSA was observed at higher essential oil concentrations, as its activity remained constant. Correspondingly, MLEs showed some activity at low concentrations but at higher concentrations (100 μg/mL) their RSA was superior to BHT and ascorbic acid at same concentrations.

Low RSA shown by oregano oil can be explained by its poor solubility in a polar medium, in such case the antioxidant activity is due to the oil fraction that gets into solution. According to Moller, Lindberg, Aaltonen, and Skibsted (1999), oregano oil constituents such as carvacrol, β-phellandrene and thymol show partial solubility in aqueous systems, which can explain limited oil solubility and relative saturation at lower concentrations (data not shown). This is understandable when considering the structure and chemical properties of the oregano oil components. It is known that oregano essential oil is composed basically of oxygenated monoterpenes such as carvacrol and thymol, which have simple phenolic structures. Based on structure–activity relationships related to antioxidant effects, it has been deduced that polymeric phenols are stronger antioxidant than monomeric phenolic compounds (Moure et al., 2001). Also catecholic functionality i.e., ortho phenolic dihydroxylation, renders higher activity than monohydroxylated phenolic compounds (Matsuura et al., 2003)

In the case of MLEs no problem with solubility was encountered, considering their hydrophilic nature. As aqueous condensates of the steam distillation process, they contained phenolic acids and flavonoids, which are well known for having good antioxidant activity (Dorman, Bachmayer, Kosar, & Hiltunen, 2004). The RSA for these extracts followed a concentration-dependent pattern as reported previously for aqueous extracts from oregano

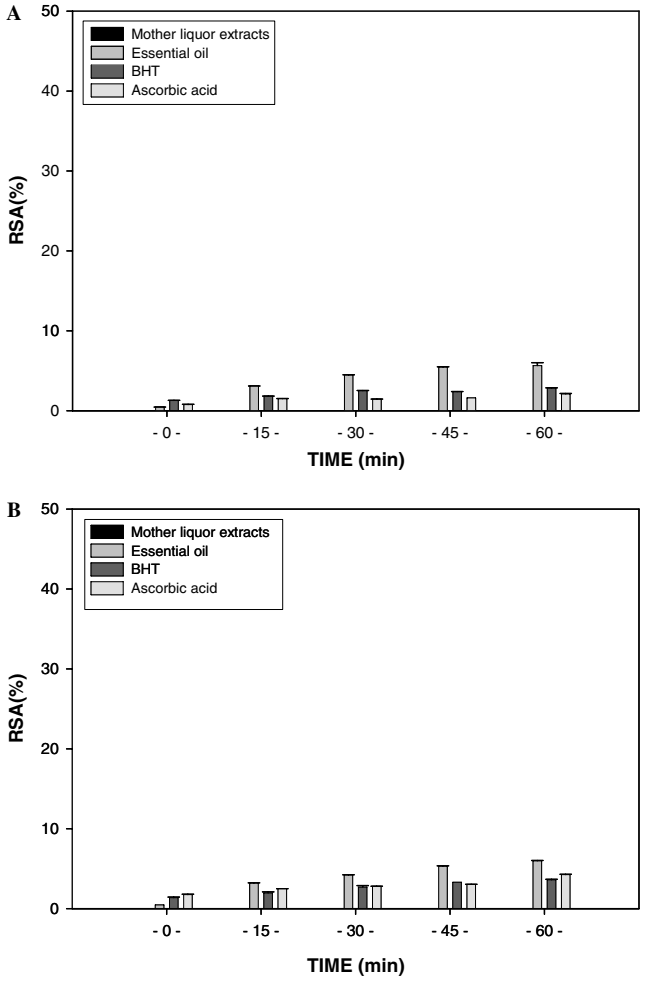

Fig. 2. Free radical scavenging activity (RSA) against DPPH* radical from oregano essential oil and controls at 1 (A) and 5 μg/mL (B) (at these concentrations mother liquor extracts did not show significant effects).

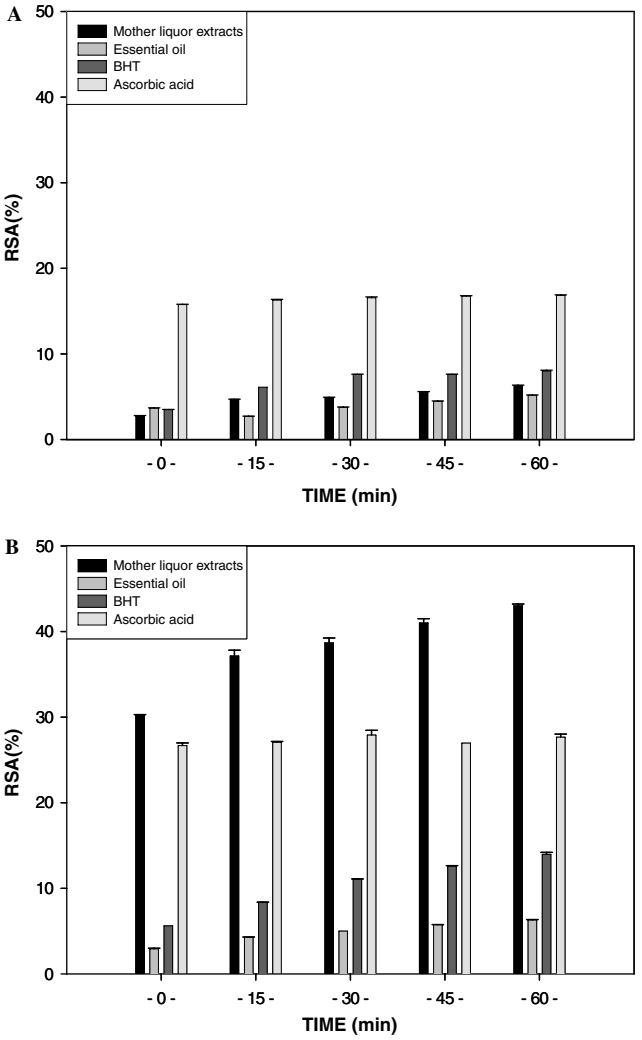

Fig. 3. Free radical scavenging activity (RSA) against DPPH* radical from oregano essential oil and mother liquor extracts at 50 (A) and 100 (B) μg/mL.

(Sokmen et al., 2004) and for phenolic acids (Nenadis, Zafiropoulou, & Tsimidou, 2003). Similarly, ethyl acetate crude extracts from aromatic plants and extracts from their distillates have shown RSA values of higher than 50% (Parejo et al., 2002). In this experiment only MLEs at 100 μg/mL showed comparable RSA values.

Deoxy-D-ribose assay results for both essential oil and MLEs showed a good scavenging activity over hydroxyl radicals at the concentrations tested. They presented RSA percentages of 45.69 ± 3.5 (at 1 μg/mL) and 50.17 ± 4.06 (at 5 μg/mL) for the essential oil and 50.95 ± 7.51 (at 50 μg/mL) and 53.88 ± 3.15 (at 100 μg/mL) for the MLEs. No significant statistical differences were found for the oil ($p = 0.070$), as well as for the MLEs ($p = 0.399$). This means that the hydroxyl RSA was similar for both samples, independent of their concentration and polarity nature. This behavior has been well documented for different polarity extracts by several researchers. Parejo et al. (2002), reported a high hydroxyl RSA by hexane, dichloromethane and ethyl acetate extracts from aromatic plants. Vardar-Ünlü, Candan, Sokmen, Donmes, and Tepe (2003) cited a 50% inhibition of hydroxyl radicals from a Lamiaceae plant essential oil at 1.40 ± 0.03 μg/mL. Also a strong hydroxyl radical stabilizing activity was documented for *O. vulgare* constituents (Lindberg & Bertelsen, 1995). Furthermore, aqueous extracts from oregano were reported as being very effective in scavenging hydroxyl radicals (Triantophyllou, Blekas, & Boskou, 2001).

From the LDL oxidation assay, solutions tested and their concentrations are listed in Table 1. Fig. 4 shows induction times estimated for both samples at the tested concentrations. Extracts showed a concentration dependency behavior. The essential oil at 1 μg/mL presented an excellent protection effect because it slowed down oxidation up to 121.19%. Although MLEs at 2.5 μg/mL could do it only by 48%, both samples delayed the induction period. This means that they are able to retard the oxidation onset, specially the oregano essential oil. However, neither oregano oil, nor MLEs were capable of inhibiting the propagation step and eventual oxidation of LDL molecules. These results are in agreement with those reported for *O. vulgare* by Dorman et al. (2003).

Response of essential oil against induced oxidation by copper ions may be explained by its lipophilic nature. This property facilitates movement of oil components into the lipoprotein (LDL), allowing them to act as scavengers of the lipid peroxyl radicals formed in the LDL particle (Milde, Elstner, & Graßmann, 2004). It has been reported (Dorman et al., 2003) that active components from extracts for scavenging lipid radicals formed in LDL particles need to be able to be incorporated inside the LDL molecule. Lipophilic character of essential oil also limits its activity at the LDL particle surface (Schwarz et al., 2000). For this reason antioxidant components in samples cannot act at the surface, breaking oxidative chain reaction and avoiding oxidation of the LDL surface.

Table 1
Sample concentrations for LDL oxidation assay

Sample	Sample concentration[a] (μg/mL)	LDL protein final concentration (mg/mL)	$CuSO_4$ concentration (μM)
Essential oil	1.0	0.1	10
	2.5	0.1	10
Mother liquor extract	1.0	0.1	10
	2.5	0.1	10

[a] Sample concentration was determined in base to total phenolic content.

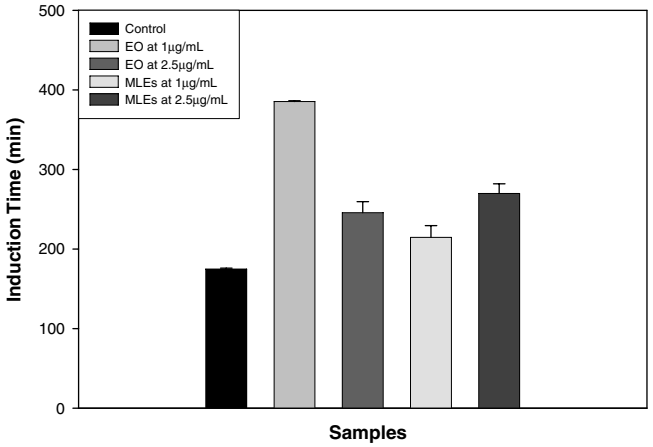

Fig. 4. Induction times shown by oregano essential oil (EO) and mother liquor extracts (MLEs) at 1 and 2.5 μg/mL. Soy oil was used as control.

Dorman et al. (2003) have documented that an increase in induction period, keeping constant the other stages in the oxidation kinetics, implies an internal mechanism of action and not a superficial one. In that sense the free radical scavenging and antioxidant effects of oregano oil occur mainly at internal level. As expected, MLEs was less efficient due to its hydrophilic nature and concentration dependency as reported for hydrophilic compounds (Anderson et al., 2001). Hydrophilic antioxidants are unable of penetrating lipid fraction of lipoproteins, therefore possess lower effectiveness compared to lipophilic antioxidants (Abuja, Murkovic, & Pfannhaoser, 1998). However, active components from MLEs can be grouped at the lipoprotein surface, acting and protecting the protein via scavenging oxygen radicals generated at the aqueous phase (Milde et al., 2004). Additionally, antioxidant effects of MLEs can be explained by a chelating mechanism of the copper ions present in the aqueous phase. Antioxidant phenolics complex metal ions, avoiding any further oxidative action of LDL molecules. This mechanism has been well documented for polar compounds such as flavonoids (Vaya et al., 2003).

4. Conclusions

No differences were found in total phenolic content of oregano essential oil and MLEs. The DPPH radical assay showed low RSA of oregano essential oil. MLEs exhibited

no significant RSA at low concentrations but at higher concentrations (100 μg/mL), was superior to the controls (ascorbic acid and BHT). Deoxy-D-ribose assay results for both essential oil and MLEs showed a good hydroxyl RSA at concentrations tested. As expected, MLEs was less efficient due to its hydrophilic nature and concentration dependency, as reported for hydrophilic compounds. However, active components from MLEs can be grouped at the lipoprotein surface, acting and protecting the proteins by scavenging oxygen radicals generated at the aqueous phase. Additionally, antioxidant effects of MLEs can be explained by a chelating mechanism of the copper ions present in the aqueous phase. Antioxidant phenolics complex metal ions, avoid any further oxidative action of LDL molecules. Solubility problems, chemical constituents, and their hydrophilic–lipophilic distribution are key factors that explain samples behavior for eventual utilization of these natural products.

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