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Full Length Research Paper

Antioxidant activities of *Rosmarinus officinalis* L. essential oil obtained by hydro-distillation and solvent free microwave extraction

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The essential oils of *Rosmarinus officinalis* L. growing in a rural area within the Nkonkobe Municipality of the Eastern Cape, South Africa, were extracted using the solvent free microwave extractor (SFME) and hydro-distillation (HD) methods. The antioxidant and free radical scavenging activity of the obtained oils were tested by means of 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^+$) assay and β-carotene bleaching test. In the $DPPH^+$ assay, while the free radical scavenging activity of the oil obtained by SFME method showed percentage inhibitions of 48.80, 61.60 and 67.00%, the HD oil showed inhibitions of 52.20, 55.00 and 65.30% at 0.33, 0.5 and 1.00 mg/ml, respectively. In the β-carotene bleaching assay, the percentage inhibition increased with increasing concentration of both oils, with a high antioxidant activity of the oil obtained through the SFME than through the HD method. The significance of this observation is discussed with respect to the properties of essential oils obtained using different methods.

Key words: Essential oil, antioxidant, hydrodistillation, solvent free microwave extraction; *Rosmarinus officinalis.*

INTRODUCTION

Plants, especially herbs and spices, have many phyto-chemicals which are potential sources of natural anti-oxidants. These include phenolic diterpenes, flavonoids, tannins and phenolic acids (Dawidowicz et al., 2006). Some antioxidants have been widely used as food additives to protect food against oxidative degradation by free radicals. Spices which are used in different types of food to improve flavors are well known for their antioxidant properties (Madsen and Bertelsen, 1995). Recently, there has been increasing interest in the use of natural antioxidants such as tocopherols, flavonoids and extracts from rosemary (*Rosmarinus officinalis* L.) for food preservation (Hras et al., 2000; Bruni et al., 2004; Williams et al., 2004; Fruitos and Hernandez-Herrero, 2005). According to these authors, these natural antioxidants do not present health problems that may arise from the use of synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG) which have side effects (Amarowicz et al., 2000; Aruoma et al., 1992). Antioxidants are compounds that neutralize chemically active products of metabolism, such as free radicals which can damage the body. It has been documented that plant phenols, with their potential to act as antioxidants, play a major role in the prevention of cancer, cardiovascular and neurogenerative diseases which are believed to be caused by oxidative stress (Losso et al., 2007).

R. officinalis L. is a perennial herb with ever-green needle like leaves that belongs to the Lamiaceae family. The Lamiaceae is a large family, rich in aromatic species that are used as culinary herbs, in folk medicines and fragrances, and many members of this family possess essential oils that are secreted by glandular trichomes (Marin et al., 2006). Previous studies have shown that rose-

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mary essential oil have antimicrobial, antioxidant, anti-carcinogenic, cognition-improving and certain glucose level lowering properties which make it useful as a natural animal feed additive (Fahim et al., 1999; Debersac et al., 2001; Fu et al., 2007).

Whilst the general antioxidant potential of *R. officinalis* essential oil have been reported before (Gachkar et al., 2007), there is no information on the possible effect of the method of extraction of the essential oils on the antioxidant property of this herb. The biological and phytochemical properties of essential oils extracted through different methods have been found to depend on the extraction method (Okoh et al., 2010). In this paper, we report the effects of solvent free microwave extraction and hydro-distillation extraction methods on the antioxidative properties of the essential oils of *R. officinalis*. The effects of these two extraction methods on the chemical compositions and antimicrobial activities of the oils have been previously reported in our laboratory (Okoh et al., 2010).

MATERIALS AND METHODS

All solvents and reagents used in these experiments such as methanol, chloroform and ethanol were of analytical grade and together with 2,2-diphenyl-2-picylhydrazyl hydrate (DPPH), 3,5-di-tert-butyl-4-hydroxytoluene (BHT) α-tocopherol, gallic acid (GA), rutin, β-carotene, linoleic acid and Tween 40, were all purchased from Sigma Aldrich (Pty) Ltd- Johannesburg, South Africa.

Collection of plant material and extraction of the essential oil

Collection, processing of the plant materials and extraction of the essential oils using SFME and HD methods were in accordance with our previous report (Okoh et al., 2010). Briefly, two samples of fresh leaves (250 g each) of *R. officinalis* were collected in February, 2009 from the vicinity of the University of Fort Hare, Alice campus in the Eastern Cape Province of South Africa (latitudes 30°00-34°15S and longitudes 22°45-30°15E). The plant was identified by Prof Don Grierson of the Botany Department, University of Fort Hare, Alice and a voucher specimen (Okoh/10) was deposited at the University herbarium.

SFME was carried out with a Milestone DryDIST (2004) apparatus. The multimode reactor has a twin magnetron (2 × 800 W, 2450 MHz) with a maximum delivered power of 500 W in 5 W increments. A rotating microwave diffuser ensures homogeneous microwave distribution throughout the plasma coated PTFE cavity. The temperature was monitored by an external IR sensor. Constant conditions of temperature and water were guaranteed by the reflux of condensed water, which was achieved by a circulating cooling system at 5°C. Two hundred and fifty grams (250 g) of the plant leaves were placed into the reactor without addition of water or any solvent. The exhaustive extraction of the essential oil was obtained in 40 min.

For the HD, 250 g of the plant leaves were hydrodistilled for 3 h in an all-glass Clevenger apparatus in accordance with the description of the British Pharmacopoeia (1980). Heat was supplied to the heating mantle (50°C) and the essential oil was extracted with 4 L of water for 3 h (until no more essential oil was released). The oils collected from both extraction methods were analyzed immediately after each collection using GC-MS. The compositions of these oils have been previously reported (Okoh et al., 2010).

Antioxidant activity

DPPH radical scavenging activity

The method of Liyana-Pathiranan and Shahidi (2005) was used for the determination of scavenging activity of DPPH free radical in the essential oils. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of oil prepared in methanol containing 0.025 to 0.5 mg of the essential oil and standard drugs (BHT and rutin). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectro-photo-metrically at 517 nm. The ability of the plant essential oils to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity = $\{(Abs_{control} - Abs_{sample})\} / (Abs\ control\} \times 100$

where, $Abs_{control}$ is the absorbance of DPPH radical + methanol, Abs_{sample} is the absorbance of DPPH radical + sample oil /standard.

β- carotene bleaching assay

This method evaluates the capacity of the oil to reduce the oxidative loss of β-carotene in a β-carotene linoleic acid emulsion (Taga et al., 1984). β-Carotene (10 mg) was dissolved in 10 ml of chloroform ($CHCl_3$). An aliquot (0.2 ml) of this solution was added into a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed using a rotary evaporator at 40°C for 5 min. Distilled water (50 ml) was slowly added to the residue with vigorous agitation, to form an emulsion. The emulsion was added to a tube containing 0.2 ml of essential oil. The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50°C for 5 min, after which the absorbance was measured again. BHT was used as the positive control. In the negative control, the essential oils were substituted with an equal volume of ethanol. The antioxidant activity (%) of the oil was evaluated in terms of the bleaching of the β-carotene using the following formula:

$\%\ inhibition = \{(At-Ct) / (C_O-Ct)\} \times 100$

where, At and Ct are the absorbances measured for the oil and control, respectively, after incubation for 5 min, and C_O is the absorbance values for the control measured at zero time during the incubation.

The oil concentration providing 50% antioxidant activity (EC_{50}) was calculated by plotting antioxidant percentage against oil concentration.

RESULTS AND DISCUSSION

The principle of antioxidant activity is based on the availability of electrons to neutralize free radicals. In this study, the antioxidant activity of *R. officinalis* oil was evaluated by two complementary tests: scavenging of $DPPH^+$ free radicals and the β-carotene bleaching test. The results are as shown in Figures 1 and 2, respectively.

The free radical scavenging activity of essential oil of *R. officinalis* obtained by SFME revealed percentage inhibitions of 48.80 , 61.60 and 67.00%, while that of the HD oil showed percentage inhibitions of 52.20, 55.00 and

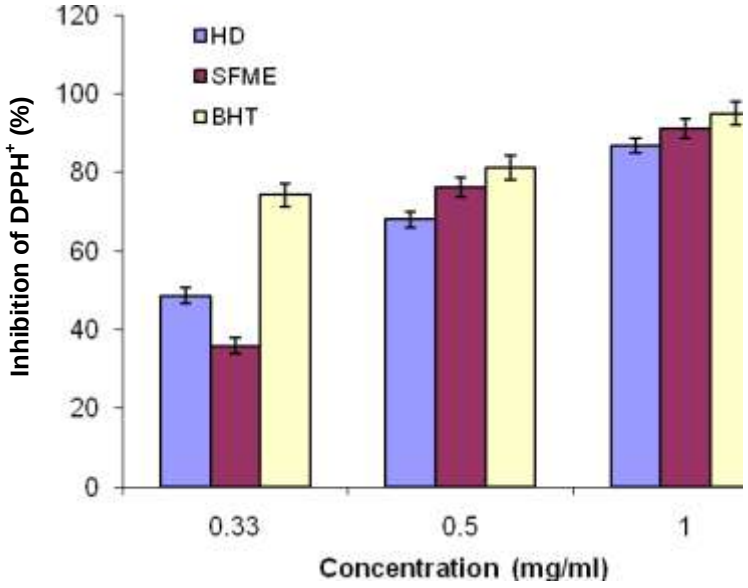

Figure 1. Free radical-scavenging activity of *R. officinalis* essential oil evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH).

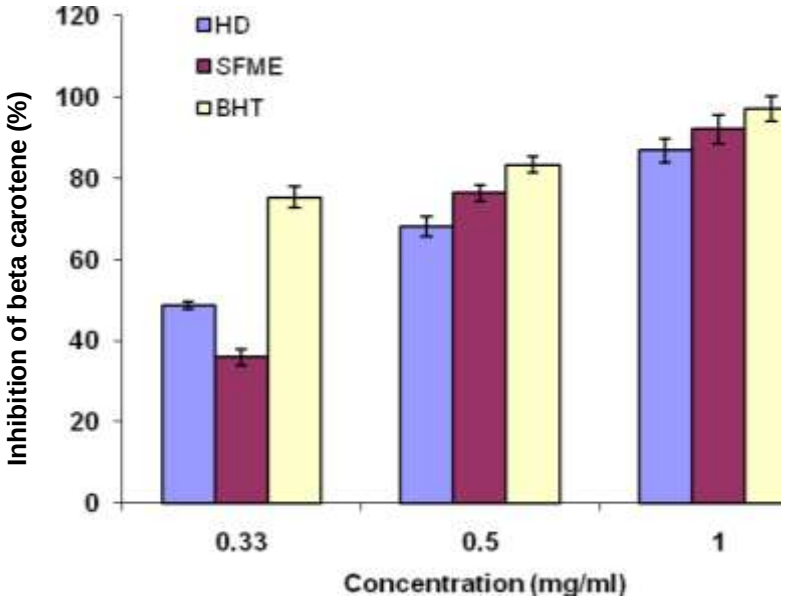

Figure 2. Antioxidant activity of *R. officinalis* L. essential oil determined by β-carotene bleaching test.

65.30% both at concentrations of 0.33 , 0.5 and 1.0 mg/ml, respectively. These results show that activity increases as the concentrations of the oils were increased, at least within the limit of the test concentrations of the oils (Figure 1). The SFME essential oil showed a slightly higher DPPH-radical scavenging activity (IC_{50} = 0.34 mg/ml) than the hydrodistilled essential oil (IC_{50} = 0.46 mg/ml), whereas for BHT, IC_{50} was 0.22 mg/ml at concentration of 0.33 mg/ml.

The result of lipid peroxidation inhibitory activity of the essentials oils, assessed by the β-carotene bleaching test are shown in Figure 2. β-Carotene usually undergoes rapid discoloration in the absence of an antioxidant. This is because the oxidation of β-carotene and linoleic acid generates free radicals (Jayaprakasha et al., 2001). The linoleic acid free radical formed upon the abstraction of a

hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecule, hence β-carotene is oxidized, losing its orange color which is then monitored spectrophotometrically (Shon et al., 2003). The results obtained from this assay are similar to the data obtained from DPPH test. The percentage inhibitions were 48.62, 68.06 and 86.79% for hydrodistilled oil; and 35.87, 76.29 and 91.19% for solvent free microwave extracted oil at concentrations of 0.33, 0.5 and 1.0 mg/ml, respectively. Although, both oils prevented the bleaching of β-carotene, the SFME extracted oil had a slightly higher activity than the HD extracted oil. These results are also consistent with the results obtained from DPPH test. The concentrations providing 50% inhibition were 0.338, 0.282, and 0.22 mg/ml for HD, SFME and BHT, respectively. It was noted that the antioxidant activities of the tested samples were dependent on their concentrations. The IC_{50} of SFME was however lower than that of HD essential oil.

In plant essential oils, oxygenated monoterpenes and monoterpene hydrocarbons are mainly responsible for the antioxidant potential (Ruberto and Baratta, 2000), and in our previous findings (Okoh et al., 2010) on the chemical constituents component of this study, we reported that oxygenated monoterpenes and monoterpene hydrocarbons were the main components of *R. officinalis* essential oil.

Many reports on the investigations of the activity of *R. officinalis* have shown that there are biologically active compounds in *R. officinalis* essential oil that exhibit cytotoxic, antioxidant, anti-carcinogenic and cognition-enhancing properties (Okamura et al., 1994; Frankel et al., 1996; Thorsen and Hildebrandt, 2003). These compounds that have the potential to influence glucose level in diabetic patients, modify rumen microbial fermentation and enhance bone desorption, but do not enhance immune response (Oluwatuyi et al. 2004; Wang et al., 2008). Essential oils, despite their wide uses and fragrances, constitute effective alternatives to synthetic compounds produced by chemical industry without showing the same side effects as the latter (Faixova and Faix, 2008). In this study, both SFME and HD extracted oil exhibited remarkable antioxidant activities. In general, the essential oil obtained from SFME showed greater activity than the one obtained from HD.

It is very difficult to attribute the antioxidant effect of a total essential oil to one or a few active principles because an essential oil always contains a mixture of different chemical compounds. In addition to the major compounds, minor molecules may make significant contributions to the oil activity. Therefore, the antioxidant property of the oils from this herb might be the combined activities of the various major and minor components of the oils.

In conclusion**,** the results of this study showed some differences in the antioxidant activities and chemical composition of the essential oils obtained by SFME and HD extraction methods. The SFME extracted oil showed a higher activity than that obtained by HD. This is probably due to the higher proportions of oxygenated compounds in SFME extracted oils. According to Okoh et al. (2010), higher amounts of oxygenated compounds with lower amounts of monoterpene hydrocarbons were present in the essential oils of this plant isolated by SFME in comparison with the oil obtained by traditional hydro-distillation.

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