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Antimicrobial, Antioxidant and Free Radical Scavenging Activities of Essential Oils Extracted from Six *Eucalyptus* Species

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Abstract*—Eucalyptus* species are well reputed for their traditional use in Asia as well as in other parts of the world; therefore, the present study was designed to investigate the antimicrobial and antioxidant activities associated with essential oils from different *Eucalyptus* species. Essential oils from the leaves of six *Eucalyptus* species, including: *Eucalyptus woodwardi, Eucalyptus stricklandii, Eucalyptus salubris, Eucalyptus sargentii, Eucalyptus torquata* and *Eucalyptus wandoo* were separated by hydrodistillation and dried over anhydrous sodium sulphate. DPPH, ferric reducing antioxidant power, and hydroxyl radical scavenging activity assays were carried out to evaluate the antioxidant potential of the oils. The results indicate that examined oils exhibit substantial antioxidant activities relative to ascorbic acid. Previously, these oils were evaluated for their antimicrobial activities, against wide range of bacterial and fungal strains, and they were shown to possess significant antimicrobial activities. In this study, further investigation into the growth kinetics of oil-treated microbial cultures was conducted. The results clearly demonstrate that the microbial growth was markedly inhibited when treated with sub-MIC concentrations of the oils. Taken together, the results obtained indicate a high potential of the examined essential oils as bioactive oils, for nutraceutical and medical applications, possessing significant antioxidant and anti microbial activities.

***Keywords*—**Antimicrobial, antioxidants, essential (volatile) oil, *Eucalyptus.*

I. Introduction

THE genus *Eucalyptus* (family *Myrtaceae*) is native to Australia and consists of more than 500 species of aromatic trees and shrubs [1]. Even though the *Eucalyptus* was formerly originated in tropical regions, today, various *Eucalyptus* species are cultivated in sub-tropical areas, as well as other climate regions. In folk medicine, *Eucalyptus* leaves have been used for wounds healing and fungal infections [1], [2]. In addition, anti- influenza and anti-malarial properties of extracts from certain *Eucalyptus* species were also reported [3]. Furthermore, local communities have used the leaves of particular *Eucalyptus* species as a folk practice for treating cancer [4]. Recently, Bardaweel et al. have reported that essential oils extracted from the leaves of numerous *Eucalyptus* species exhibited noticeable anticancer properties, with particular potency against Lymphoma tumors [5].

The expanding doubt of harmful synthetic additives, in food and pharmaceutical industries, widely contributes to the growing consumers' enthusiasm to ingredients coming from natural sources [6]. Accordingly, the deliberate use of plants essential oils as purposeful ingredients in foods, drinks, and cosmetics, is achieving enormous recognition. Owing to their bioactive constituents, essential oils have certainly shown potential in view of their use as effective antibacterial, antifungal, and antioxidant agents. With the thriving interest in essential oils, in both food and pharmaceutical industries, appraisal of the plants essential oils has become progressively more valuable [7].

Considering the diversity of *Eucalyptus* genus and the finite number of studies available on its species, there is very limited data attainable to support the biological activities associated with the various species. The current study emphasizes on the evaluation of the biological activities of the essential oils obtained from the leaves of six, previously unexplored, *Eucalyptus* species including: *Eucalyptus woodwardii* Maiden, *Eucalyptus stricklandii* Maiden, *Eucalyptus salubris* F. Muell., *Eucalyptus sargentii* Maiden, *Eucalyptus torquata* Luehm., and *Eucalyptus wandoo* Blakely.

II. Methods

A. Plant Material

Leaves of *E. woodwardii, E. stricklandii, E. salubris, E. sargentii, E. torquata* and *E. wandoo* were collected from the trees cultivated in The Jordan University of Science and Technology, Irbid, Jordan, in April, 2013. The leaves were air-dried, stored in double-layer paper bags at room temperature, and protected from the direct light. The taxonomic identity of the collected plant was confirmed by Eng. Mohammad Al-Gharaibeh, a botanist, Department of Natural Resources and Environment, Faculty of Agriculture, Jordan University of Science and Technology (JUST), Irbid, Jordan. A voucher specimen (No. KT-EU-JOR-2013) has been deposited at the herbarium of the same institute.

B. Essential Oil Isolation

The essential oils of fresh leaves of *E. woodwardii, E. stricklandii, E. salubris, E. sargentii, E. torquata* and *E. wandoo* were individually attained by hydrodistillation. The leaves were dried and then distilled using a Clevenger type apparatus for 6 h. The essential oils were dried with anhydrous

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International Science Index, Biotechnology and Bioengineering Vol:9, No:1, 2015 waset.org/Publication/10000545

sodium sulphate and stored in glass vial with Teflon-sealed caps at 4◦C in the absence of light until used.

C. Antioxidant Activity

1) DPPH Free Radical Scavenging Activity

The free-radical scavenging activity of the hydrodistilled oil was measured as a decrease in the absorbance of methanol solution of 2,2-diphenyl-1-picrylhydrazil (DPPH). A stock solution of DPPH (0.1mM) was prepared in methanol and different concentrations of the essential oils were individually added (5-250 µg/ml). After incubation at room temperature for 30 min, the pale pink color developed was measured spectrophotometerically at 517 nm and compared with the standard (5-250 µg/ml ascorbic acid) [8]. Free radical scavenging activity was expressed as the percentage inhibition calculated using the following formula:

% Anti-radical activity = (Control Absorbance- Sample Absorbance) × 100/Control Absorbance

2) Hydroxyl Radical Scavenging Activity

Each oil (0.2 ml) at different concentrations (5-250 µg/ml), was added to 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) and 0.5 ml of 0.22% ascorbic acid were added to each tube. The tubes were capped tightly and heated in a water bath at 80-90°C for 15 min. The reaction was terminated by adding 1 ml of ice-cold trichloroacetic acid (TCA) (17.5% w/v). Afterward, 3 ml of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and distilled water was added to make up total volume of 1 L) was added to each tube, then left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured at 412 nm against blank [9]. Percentage inhibition was determined by comparing the results of the test and the standard compound (ascorbic acid) by using the formula:

% inhibition activity = (Control Absorbance- Sample Absorbance) × 100/Control Absorbance

3) Ferric-Reducing Antioxidant Power (FRAP) Assay

The reducing power of the oils was determined by the Ferric-Reducing Antioxidant Power (FRAP) assay described by Yen and Chen [10]. One ml of different concentrations of the oil (5-250 µg/ml) was mixed with 2.5 ml of potassium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 g/100 ml). The mixture was incubated at 50°C for 20 minutes. TCA (10%, 2.5 ml) was added to the mixture to terminate the reaction. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride. The procedure was repeated in triplicate and allowed to stand for 30 min before measuring the absorbance at 700 nm. Ascorbic acid was used as a positive control. The percentage of antioxidant activity in FRAP assay of the samples was calculated according to the formula:

Antioxidant Activity (%) = (A1A0)/A1

where, A0 = Absorbance of the control (potassium phosphate buffer + FRAP reagent), A1 = Absorbance of sample.

D. Growth Kinetics

The growth kinetics of representative bacteria and fungi were determined in the presence of sub-MIC concentrations of the examined oils individually. Briefly, stationary-phase cultures were prepared and used to inoculate 200 mL of MHB broth (for bacteria) or YPD broth (for fungi) to an initial OD_{600} of 0.05. These cultures were divided into 10.0-mL aliquots and each of the tested oils was added to yield the desired concentration. A growth curve of the tested microorganism, metabolizing the essential oil added to the culture, was constructed by monitoring OD_{600}. The test flasks were shaken at 37ºC and samples were drawn at each time point for $OD6_{00}$ readings until the stationary phase was reached. Growth kinetic assays for each microorganism were performed in triplicate from the same stationary-phase starter culture. Triplicate growth curves were then repeated three times using independent stationary-phase starter cultures.

III. Results And Discussion

An antioxidant is described as a substance that substantially suspends or inhibits an oxidation process [11]. The antioxidant activity is frequently determined via measuring the inhibition rate of an oxidation processes in the presence of an antioxidant [11]. Antioxidant efficiency is oftentimes associated with the antioxidant capability to scavenge stable free radicals. DPPH is largely used to assess the antiradical activity of a given compound or extract. Table I indicates the free radical scavenging activities of the examined essential oils, at a concentration of 100 µg/ml, relative to ascorbic acid, at the same concentration. As depicted, the oils possess moderate to significant radical scavenging activities relative to the strong antioxidant ascorbic acid (p-value <0.05), which demonstrates 85% radical scavenging activity at 100 µg/ml.

TABLE I
Antioxidant and Free Radical Scavenging Activities

Antioxidant	DPPH inhibition %	OH-radical scavenging %	Fe-reducing %
E.woodwardi	32	36	28
E.stricklandii	51	54	47
E.salubris	44	42	39
E.sargentii	67	64	66
E. torquata	35	33	26
E.wandoo	59	54	61
Ascorbic acid	85	84	89

Additionally, the ability of the oils to reduce iron (III) to iron (II) was evaluated and compared to ascorbic acid, as shown in Table I. The results illustrate that some of the examined essential oils, specifically the *E. wandoo* and *E. sargentii*, were capable to reduce iron in a manner that is nearly comparable to the ability of ascorbic acid reduction efficiency of the sample (Table I), at the same concentration

International Science Index, Biotechnology and Bioengineering Vol:9, No:1, 2015 waset.org/Publication/10000545

(p-value <0.05).

Furthermore, the hydroxyl (OH^-) reactive oxygen species (ROS) is one of the most reactive and physiologically destructive free radicals. In this study, 100 µg/ml concentration of the essential oils was, individually, needed for scavenging 36-64% of the hydroxyl activity, whereas the standard antioxidant, ascorbic acid, was able to result in 84% scavenging of the hydroxyl activity, at 100 µg/ml (Table I). Apparently, the scavenging activity possessed by the essential oil of *E.sargentii* is proportionate to that associated with the strong antioxidant ascorbic acid (p-value <0.05).

Free radicals are the most prevalent initiators of oxidative reactions that may generate deleterious effects [11]. Natural antioxidants can scavenge and react with free radicals, and hence abolish the free radical reaction. Lately, herbal medicines possessing free radical scavenging potential are gaining special attention from the pharmaceutical research for their value in impeding and treating several diseases and disorders [12]. Typically, essential oils were shown to protect against oxidative stress by contributing to the total antioxidant defense system of the human body [13]. Recently, a number of essential oils isolated from several medicinal and aromatic plants were found to possess significant antioxidant potential and, therefore, provide a safeguard against some cardiovascular and degenerative diseases [13], [14]. Our findings verify that the examined essential oils have considerable antioxidant potential that may be attributed to their chemical composition. Ongoing research should reveal the active chemical components of the oils that are responsible for their antioxidant activities.

Previously, Bardaweel et al. reported that the essential oils obtained from the leaves of *E. woodwardii*, *E. stricklandii, E. salubris, E. sargentii, E. torquata* and *E. wandoo* were shown to exhibit substantial antimicrobial properties against clinically problematic bacteria and fungi (Table II) [5]. In this report, the growth kinetics of *Escherichia coli* and *Candida albicans* growing in the presence of sub-MIC concentrations of the examined oils were studied. Growth curves for the two examined microorganisms reveal typical sigmoidal kinetics with identifiable lag and logarithmic growth phases (only data for a representative oil were shown). Duration of the lag and log phases vary among the two examined microorganisms. Generally, about 3 to 7 h were needed for the cells to adjust to the normal growth environment before they were ready to proliferate and hit the log phase. The patterns of growth curves of the examined microorganisms were altered and showed deviations from the normal curves following treatment with any of the essential oils used in this study (only data for *E. sergentii* is shown in Fig. 1). Growth of the microorganisms was severely inhibited when treated with sub-MIC concentrations of the oils. Specifically, a clear shift to the right due to extension of the lag phase was observed in all treated cultures.

TABLE II
MIC VALUES AGAINST REPRESENTATIVE MICROBES

Essential oil	*Escherichia coli**	*Candida albicans**
E.woodwardi	178	196
E.stricklandii	183	169
E.salubris	119	95
E.sargentii	110	76
E. torquata	204	192
E.wandoo	158	103

*MIC Values are expressed in ug/ml and were acquired from [5]. Results represent the means of three independent readings. SD values are less than 5%.

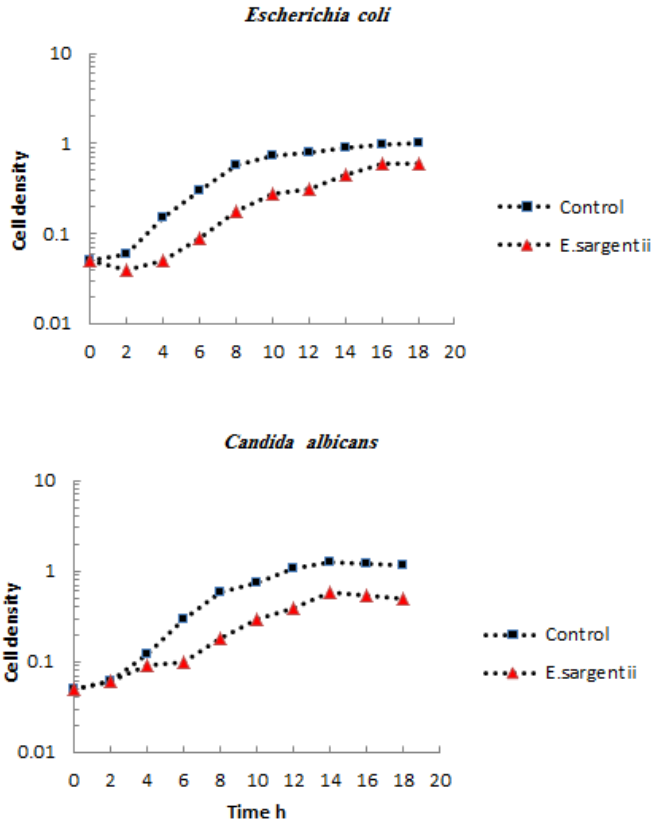


Fig. 1 Growth curves of representative bacteria and Candida cultures treated with sub-MIC concentrations of *E. sergentii*. The values are expressed mean ± SD from three independent experiments performed in triplicate (n = 9). Standard deviation values were less than 5%.

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