Bioresource Technology 101 (2010) 3751–3754

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract

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ARTICLE INFO

Article history:
Received 21 August 2009
Received in revised form 10 December 2009
Accepted 15 December 2009
Available online 27 January 2010

Keywords:
Olive leaf extract
Phenolic compounds
Antioxidant activity
Antimicrobial activity
Individual and combined phenolics

ABSTRACT

Olive leaves, an agricultural waste, have great potential as a natural antioxidant. The current study was made to assess the antioxidant and antimicrobial activities of both the individual and combined phenolics in olive leaf extract. A combined phenolics mixture was prepared by amount ratios of the phenolic compounds in the olive leaf extract. The results showed that both the individual and combined phenolics exhibited good radical scavenging abilities, and also revealed superoxide dismutase (SOD)-like activity. In terms of antimicrobial activity, both oleuropein and caffeic acid showed inhibition effects against microorganisms. Furthermore, the antimicrobial effect of the combined phenolics was significantly higher than those of the individual phenolics. These results show that the combination of olive leaf extract phenolics possessed antioxidant and antimicrobial activities. This study indicates that olive leaf extract might be a valuable bioactive source, and would seem to be applicable in both the health and medical food.

1. Introduction

Olive (*Olea europaea*) leaf has been widely used in folk medicine for several thousand of years within European Mediterranean islands and countries (Gucci et al., 1997). Historically, olive leaf was used for the treatment of malaria and associated fever (Benavente-Garcia et al., 2000). The major physiological substances of olive leaf are hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid, vanillic acid, vanillin, oleuropein, luteolin, diosmetin, rutin, verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside (Bianco and Uccella, 2000; Tasioula-Margari and Ologeri, 2001).

Several reports have been published on olive leaf and presented the following: olive leaf offered a capacity to lower blood pressure and increase blood flow in the coronary arteries (Khayyal et al., 2002), the phenolic compounds extracted from olive leaf possessed antioxidative activity (Somova et al., 2003; Skerget et al., 2005) as well as antimicrobial activity against *Helicobacter pylori*, *Campylobacter jejuni*, *Staphylococcus aureus* (Sudjana et al., 2009), the optimum extraction conditions for the supercritical fluid extraction of olive leaf polyphenols were examined (Floch et al., 1998), and the nutritive value of olive leaf was assessed according to certain cultivars (Delgado-Pertinez et al., 2000). In addition, we previously reported that olive leaf contains significant amounts of oleuropein and phenolics, important factors for its antioxidant capacity (Lee et al., 2009). Moreover, Benavente-Garcia et al. (2000) reported that olive leaf extract showed a synergic behavior, characteristic of its high content of oleuropein and other active phenolics.

Despite the many reports on olive leaf and its phenolic compounds, the combined effects of olive leaf phenolics in terms of antioxidant and antimicrobial activities have not been studied. Additionally, the demands for olive leaf and olive leaf extract have been increasing for utilization as food materials, food additives, and supplements. Therefore, the aim of this study was to provide basic data on the antioxidant and antimicrobial activities of both the individual and combined phenolics in olive leaf extract to predict the usefulness of olive leaf as a functional food ingredient. The antioxidant and antimicrobial potentials of olive leaf extract were assessed by nitrite-scavenging ability, SOD-like and DPPH radical scavenging activities, along with its inhibitory activities against Gram-positive and negative microorganisms.

2. Methods

2.1. Materials

Dimethyl sulfoxide (DMSO), caffeic acid, vanillin, and rutin were purchased from Sigma (St. Louis, MO, USA), and oleuropein was obtained from Extrasynthese (Genay, France). All other chemicals were reagent grade. The extraction and preparation of olive leaf extract were performed as described previously (Lee et al., 2009). A combined phenolics mixture was prepared by the amount ratios of the phenolic compounds in olive leaf extract (oleuropein:rutin:vanillin:caffeic acid = 100:1.35:0.65:0.30, w/w).

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doi:10.1016/j.biortech.2009.12.052

2.2. In vitro antioxidant and antimicrobial activities

The in vitro antioxidant activities of both the individual and combined phenolics were determined by nitrite-scavenging ability, SOD-like and DPPH radical scavenging activities, along with the assessment of their antimicrobial activities. Nitrite-scavenging ability was determined by the method of Gray and Dugan (1975). A sample amount of 1 mL and 1 mL of 1 mM $NaNO_2$ were dissolved in 0.1 N HCl (pH 1.2) for a total of 10 mL. After incubating at 37 °C in a water bath for 2 h, 1 mL of reaction solution was combined with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent in test tubes and vortexed and kept at room temperature for 15 min. The amount of residual nitrite in the reaction mixture was measured using a spectrophotometer at 520 nm. Nitrite-scavenging ability was calculated as follows: nitrite-scavenging activity (%) = $[1 - ((A - C)/B)] \times 100$, ($A$; absorbance of 1 mM $NaNO_2$ added sample after standing for 1 h, B; absorbance of 1 mM $NaNO_2$, C; absorbance of control). The SOD-like and DPPH radical scavenging activities were assayed as described previously (Lee et al., 2009).

The strains of microorganisms employed were the Gram-positive bacteria, *Bacillus cereus* (KCCM 40935) and *S.* aureus (KCCM 40907), and the Gram-negative bacteria, *Escherichia coli* (KCCM 11234) and *Salmonella enteritidis* (KCCM 12021). The individual and combined phenolics in the olive leaf extract were dissolved in 1% DMSO and sterilized by filtration through 0.45 μm Millipore filters. The antimicrobial activities were then examined by the paper disc diffusion method (Kim et al., 2000) using 100 μL of suspension containing 10^8 CFU/mL of bacteria spread onto nutrient agar medium. The 8-mm diameter paper discs were impregnated (800 μg/disc) with 20 μL of each sample and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the phenolic compounds in the olive leaf extract. The inoculated plates were incubated at 37 °C for 24 h for clinical bacterial strains. The antimicrobial activities were evaluated by measuring the zones of inhibition against the test organisms.

2.3. Statistical analysis

All measurements were repeated three times. The results are expressed as mean values and standard deviations. The data were statistically analyzed by ANOVA and Duncan's multiple range tests. Statistical significance was accepted at a level of $P < 0.05$ (SAS Institute, 1988).

3. Results and discussion

3.1. Antioxidant activities

The human health risk associated with exposure to nitrate is considered to be methemoglobinemia due to endogenous conversion of nitrate to nitrite. Nitrite derived from nitrate may react in vivo with amine and amides to form *N*-nitroso compounds, which may have carcinogenic properties. It is also present in large quantities in meat coloring agents and processed meats (van Maanen et al., 1996). Therefore, we evaluated the potential chemopreventive effects of the individual and combined phenolics in olive leaf extract using the nitrite-scavenging method. The nitrite-scavenging abilities of oleuropein, rutin, vanillin, caffeic acid, and their mixture at 500 μM were 72.7%, 47.8%, 6.3%, 92.2%, and 66.6%, respectively. At 1000 μM, these values were increased for 86.8%, 88.1%, 11.3%, 97.8%, and 84.8% (Fig. 1A). All the phenolic compounds (except vanillin) in the olive leaf extract showed strong nitrite-scavenging abilities, as both individual and combined phenolics. These results suggest that the phenolic compounds in

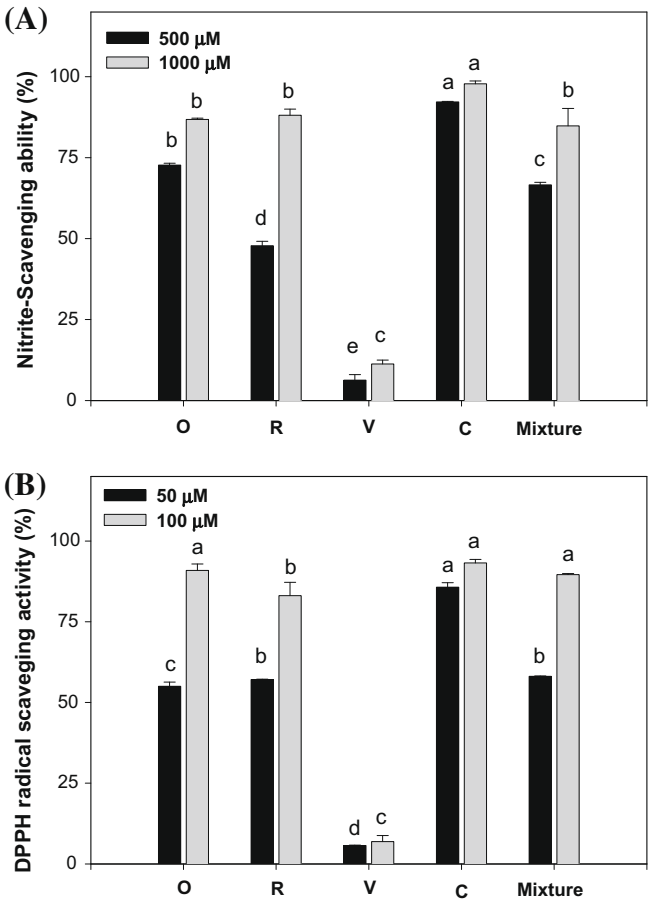

Fig. 1. Nitrite (A) and DPPH radical (B) scavenging abilities of individual and combined phenolics in olive leaf extract. O: oleuropein, R: rutin, V: vanillin, C: caffeic acid, mixture; mixture was prepared by amount ratios of phenolic compounds in the olive leaf extract: oleuropein, rutin, vanillin, caffeic acid (100:1.35:0.65:0.30, w/w). [a–c]Means in the same column not sharing a common letter are significantly different ($p < 0.05$) by Duncan's multiple test.

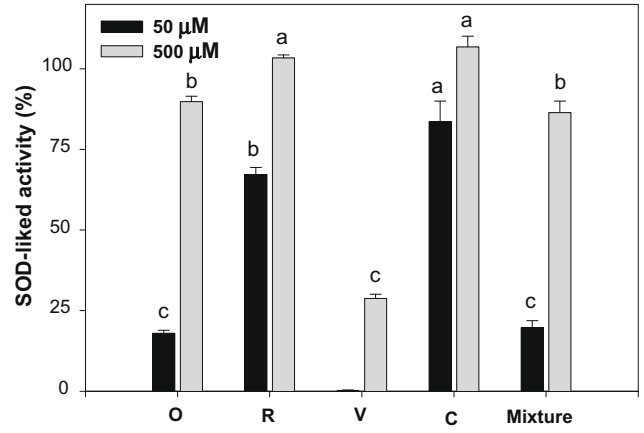

Fig. 2. SOD-like activity of individual and combined phenolics in olive leaf extract. O: oleuropein, R: rutin, V: vanillin, C: caffeic acid, mixture; mixture was prepared by amount ratios of phenolic compounds in the olive leaf extract: oleuropein, rutin, vanillin, caffeic acid (100:1.35:0.65:0.30, w/w). [a–c]Means in the same column not sharing a common letter are significantly different ($p < 0.05$) by Duncan's multiple test.

Table 1
Antimicrobial activities of oleuropein, rutin, vanillin, caffeic acid, and their mixture[a] in olive leaf extract.

Microorganisms tested	Conc. (g/disc)	Oleuropein (mm)	Rutin (mm)	Vanillin (mm)	Caffeic acid (mm)	Mixture (mm)
Bacillus cereus KCCM 40935	800	–[b]	–	–	9.8 ± 0.0[b]	28.5 ± 0.3[a]
Staphylococcus aureus KCCM 40307	800	–	–	–	–	–
Escherichia coli KCCM 11234	800	–	–	–	10.1 ± 0.1	–
Salmonella enteritidis KCCM 12021	800	23.5 ± 0.8[b]	–	–	10.4 ± 0.2[c]	24.5 ± 0.5[a]

[a–c] Means in the same column not sharing a common letter are significantly different ($p < 0.05$) by Duncan's multiple test.
[a] Mixture was prepared by amount ratios of phenolic compounds in the olive leaf extract: oleuropein, rutin, vanillin, caffeic acid (100:1.35:0.65:0.30, w/w).
[b] Not detected.

olive leaf can protect against damage from nitrite and nitrosamine-related cancer. Thus, olive leaf has potential chemopreventive benefits against major oxidation-linked diseases and can be part of a healthy diet. Oxidative stress is involved in the pathology of oxidation-linked diseases such as cancer, heart disease, atherosclerosis, and rheumatoid arthritis, and may play a role in neurodegenerative diseases and aging processes (Briante et al., 2003). Dietary phenolic compounds have generally been considered as non-nutrients, and their possible benefits to human health through their phenolic-linked antioxidant effects have just recently been considered.

The DPPH radical scavenging activities (Fig. 1B) at 50 μM were in the order of caffeic acid (85.7%) > phenolics mixture (58.1%) ⩾ rutin (57.0%) > oleuropein (55.0%) > vanillin (5.8%). These results are in good agreement with a report by Briante et al. (2003), who reported that phenolic compounds can be active as antioxidants by a number of potential pathways. The most important is likely to be by free radical scavenging in which the phenol can break the free radical chain reaction. The presence of different substituent within the backbone structure of phenols modulates their antioxidant properties, in particular, their hydrogen-donating capacity.

Fig. 2 shows the SOD-like activity of the individual and combined phenolics in olive leaf extract. The SOD-like activities of oleuropein, rutin, caffeic acid, and their mixture at 500 μM were 18.0%, 67.2%, 83.6%, and 19.7%, respectively. However, no SOD-like activity was detected for vanillin. In addition, the SOD-like activities of oleuropein, rutin, vanillin, caffeic acid, and their mixture at 500 μM were 89.8%, 103.4%, 28.8%, 106.8%, and 86.4%, respectively. A significant difference was detected in which rutin and caffeic acid showed higher SOD-like activities than vanillin ($p < 0.05$). These results were similar to the nitrite-scavenging ability data, where all the phenolics (except vanillin) in the olive leaf extract showed strong nitrite-scavenging abilities as both the single and combined form.

3.2. Antimicrobial activity

Table 1 shows the antimicrobial activities and synergistic effects of the individual and combined phenolics in olive leaf extract. Oleuropein (800 μg/disc) showed a strong growth inhibition effect (23.5 mm) against *S. enteritidis*, and caffeic acid showed moderate growth inhibition effects (9.8–10.4 mm) against *B. cereus*, *E. coli*, and *S. enteritidis*. However, oleuropein and caffeic acid did not show antimicrobial activities against *S. aureus*. Consistently, no antimicrobial activities were observed for rutin and vanillin against *B. cereus*, *E. coli*, *S. enteritidis*, and *S. aureus*. Interestingly, the combined phenolics mixture showed better inhibition effects against *B.* cereus and *S. enteritidis*. A significant difference was detected in which the phenolics mixture presented higher inhibition effects than the individual phenolics ($p < 0.05$). These results indicate that oleuropein, rutin, vanillin, and caffeic acid contained in olive leaf extract offer certain synergistic effects in the mixed form.

In conclusion, although the individual phenolic compounds in olive leaf extract showed strong in vitro activities, the antioxidant and antimicrobial activities of the combined phenolics showed similar or better effects than the individual phenolics. Therefore, these results suggest that olive leaf extract has great potential as a functional food ingredient, particularly as a source of phenolic compounds. In addition, if olive leaf is to be utilized as a functional food material, a more cost-effective and safer extraction method may be to prepare crude olive leaf extract versus using purification methods such as organic solvent fraction and column chromatography.

Acknowledgement

The authors would like to thank Mr. James Kwon from Medifood for providing the olive leaf.

References

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A., Del Rio, J.A., 2000. Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chem. 68, 457–462.
Bianco, A., Uccella, N., 2000. Biophenolic components of olives. Food Res. Int. 33, 475–485.
Briante, R., Febbraio, F., Nucci, R., 2003. Antioxidant properties of low molecular weight phenols present in the mediterranean diet. J. Agric. Food Chem. 51, 6975–6981.
Delgado-Pertinez, M., Gomez-Cabrera, A., Garrido, A., 2000. Predicting the nutritive value of the olive leaf (*Olea europaea*): digestibility and chemistry composition and in vitro studies. Animal Feed Sci. Technol. 87, 187–201.
Floch, F.L., Tena, M.T., Rios, A., Valcarcel, M., 1998. Supercritical fluid extraction of phenol compounds from olive leaves. Talanta 46, 1123–1130.
Gray, J.I., Dugan, J.L.R., 1975. Inhibition of *N*-nitrosamine formation in model food system. J. Food Sci. 40, 981–985.
Gucci, R., Lombardini, L., Tattini, M., 1997. Analysis of leaf water relations in leaves of two olive (*Olea europaea*) cultivars differing in tolerance to salinity. Tree Physiol. 17, 13–21.
Khayyal, M.T., El-Ghazaly, M.A., Abdallah, D.M., Nassar, N.N., Okpanyi, S.N., Kreuter, M.H., 2002. Blood pressure lowering effect of an olive leaf extract (*Olea europaea*) in L-NAME induced hypertension in rats. Arzneimittelforschung 52, 797–802.
Kim, M.S., Lee, D.C., Hong, J.E., Chang, K.S., Cho, H.Y., Kwon, Y.K., Kim, H.Y., 2000. Antimicrobial effects of ethanol extracts from Korean and Indonesian plants. Korean J. Food Sci. Technol. 32, 949–958.
Lee, O.H., Lee, B.Y., Lee, J., Lee, H.B., Son, J.Y., Park, C.S., Shetty, K., Kim, Y.C., 2009. Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. Bioresour. Technol. 100, 6107–6113.
SAS Institute, 1988. SAS User's Guide. SAS Institue Inc., Cary, NC.
Skerget, M., Kotnik, P., Hadolin, M., Hradolin, A.R., Simoni, M., Knez, Z., 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem. 89, 191–198.
Somova, L.I., Shode, F.O., Ramnanan, P., Nadar, A., 2003. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies africana leaves. J. Ethnopharmacol. 84, 299–305.
Sudjana, A.N., D'Orazio, C., Ryan, V., Rasool, N., Ng, J., Islam, N., Riley, T.V., Hammer, K.A., 2009. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. Int. J. Antimicrob. Agents 33, 461–463.

Tasioula-Margari, M., Ologeri, O., 2001. Isolation and characterization of virgin olive oil phenolic compounds by HPLC/UV and GC/MS. J. Food Sci. 66, 530–534.
van Maanen, J.M., Welle, I.J., Hageman, G., Dallinga, J.W., Mertens, P.L., Kleinjans, J.C., 1996. Nitrate contamination of drinking water: relationship with HPRT variant frequency in lymphocyte DNA and urinary excretion of *N*-nitrosamines. Environ. Health Perspect. 104, 522–528.