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Short communication

Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract

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ABSTRACT

The aim of this research was to investigate the activity of a commercial extract derived from the leaves of *Olea europaea* (olive) against a wide range of microorganisms ($n = 122$). Using agar dilution and broth microdilution techniques, olive leaf extract was found to be most active against *Campylobacter jejuni*, *Helicobacter pylori* and *Staphylococcus aureus* [including meticillin-resistant *S. aureus* (MRSA)], with minimum inhibitory concentrations (MICs) as low as 0.31–0.78% (v/v). In contrast, the extract showed little activity against all other test organisms ($n = 79$), with MICs for most ranging from 6.25% to 50% (v/v). In conclusion, olive leaf extract was not broad-spectrum in action, showing appreciable activity only against *H. pylori*, *C. jejuni*, *S. aureus* and MRSA. Given this specific activity, olive leaf extract may have a role in regulating the composition of the gastric flora by selectively reducing levels of *H. pylori* and *C. jejuni*.

1. Introduction

Olive leaf extract (OLE) is a dark brown, bitter-tasting liquid derived from the leaves of the olive tree (*Olea europaea* L., Oleaceae). OLE is marketed as a natural medicine with wide-ranging health benefits. Particular emphasis is placed on the antioxidant activity of the extract and the corresponding health benefits such as cardioprotective and chemopreventive effects [1]. OLE contains many different compounds collectively termed olive biophenols, which are thought to give the extract its varied therapeutic properties. The most abundant biophenol is oleuropein, with other biophenols such as verbascoside, apigenin-7-glucoside and luteolin-7-glucoside present in lower quantities [2]. Like many natural products, variation due to differences such as geographical location, plant nutrition and cultivar can influence the composition of the extract.

In addition to the health benefits described above, it is claimed that OLE may aid in the treatment of a broad range of infectious diseases if ingested. Although the antimicrobial activity of the component oleuropein has been examined previously [3,4], very few reports describe the activity of the entire extract [5]. Therefore, the aim of this research was to examine the activity of OLE against a wide range of microorganisms to determine the spectrum of activity of the extract and to provide data either to support or dispute the claims made for the product.

2. Materials and methods

2.1. Organisms

Reference strains ($n = 34$) and clinical isolates ($n = 88$) were obtained from the Division of Microbiology and Infectious Diseases, PathWest Laboratory Medicine WA, the Discipline of Microbiology and Immunology at The University of Western Australia, and Royal Perth Hospital, Perth, Western Australia.

2.2. Olea europaea (olive) leaf extract

'High Strength Olive Leaf Extract' (natural flavour), by Olive Leaf Australia (Coominya, Australia), was purchased from a local health food store. According to the label, the OLE was produced in accordance with the Australian Code of GMP (Good Manufac-

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turing Practice) (AUST. L 108980) and the guaranteed minimum oleuropein content was 4.4 mg/mL.

2.3. Antimicrobial susceptibility assays

The antimicrobial activity of the extract was determined for the majority of organisms using the broth microdilution assay following the methods described by the Clinical and Laboratory Standards Institute for bacteria [6] and yeasts [7]. In addition, the following modifications were made to the methods to allow for the growth of fastidious test organisms. For streptococci, 2.5% lysed horse blood was added to the Mueller–Hinton broth (MHB). For *Listeria*, tests were conducted in cation-adjusted MHB. For lactobacilli, inocula were prepared by suspending growth from 3-day cultures on Rogosa agar grown at 35 °C with 5% CO_2, and microdilution assays were conducted in de Man, Rogosa and Sharpe (MRS) broth also incubated for 3 days at 35 °C with 5% CO_2. An agar dilution method was used to test OLE against *Helicobacter* and *Campylobacter* using Mueller–Hinton agar with 5% sheep blood and incubating plates for 4 days at 35 °C with 10% CO_2. OLE was tested in doubling dilutions ranging from 50% to 0.002% (v/v) for broth assays and from 10% to 0.016% (v/v) for tests in agar. The OLE was completely soluble both in solid and liquid growth media and as such no emulsifying agent was required. Minimum inhibitory concentrations (MICs) were determined as the lowest concentration of OLE resulting in an optically clear microtitre tray well or no growth on the agar plate. Minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs) were determined from broth microdilution assays by subculturing 10 μL volumes from each non-turbid well and spot inoculating onto an appropriate growth medium. Following incubation of plates, the number of colonies was counted. MBCs/MFCs were defined as the lowest concentration killing ≥99.9% of the inoculum compared with initial viable counts. Tests were repeated at least three times and modal MIC and MBC/MFC values were selected. MICs could not be determined visually for the streptococci as there was an interaction between the OLE and the 2.5% lysed horse blood in the medium that made all the wells turbid after 24 h incubation. MICs were therefore determined by subculture (as described above) as the lowest concentration resulting in the maintenance of, or a reduction in, the number of organisms in the inoculum.

3. Results and discussion

The organisms least susceptible to OLE, with one or more isolates having MICs of ≥50% OLE, were *Bacillus subtilis*, *Candida* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Serratia marcescens* (Table 1). Conversely, the most susceptible organism was *Campylobacter jejuni*, with MICs as low as 0.31%, followed by *Helicobacter pylori* with MICs of 0.62% and *Staphylococcus aureus* with MICs of 0.78%. The data indicate that OLE does not show broad-spectrum activity and has appreciable activity only against *C. jejuni*, *H. pylori* and *Staphylococcus* spp. The susceptibility of the closely related organisms *H. pylori* and *C. jejuni* to OLE is not surprising given that these organisms are frequently susceptible to antimicrobial agents in vitro. Furthermore, a previous study has shown *C. jejuni* isolates to be comparatively more susceptible to various natural compounds than other organisms such as *E. coli*, *Salmonella* and *Listeria* [8]. This was attributed to their fastidious nature and possibly to differences in the outer membrane and cell wall compared with the other organisms [8]. However, the susceptibility of an organism to an antimicrobial agent in vitro does not always translate into clinical efficacy, especially for *H. pylori*. Only a limited selection of agents is known to be effective at eradicating *H. pylori* from the gut in vivo [9] and further studies are therefore required.

With regard to the antistaphylococcal activity, it is possible that one or more of the components within OLE act specifically against the Gram-positive cell wall, with the staphylococcal cell wall being particularly susceptible. However, there do not appear to be any similar reports in the literature either for whole OLE or individual

Table 1
Susceptibility data for microorganisms (n = 122) tested against olive leaf extract (% v/v).

Organism (n)	MIC_{range}	MIC_{90}	MBC/MFC_{range}	MBC_{90}/MFC_{90}
Acinetobacter calcoaceticus (2)	25		25	
Bacillus cereus (1)	12.5		12.5	
Bacillus subtilis (1)	50		50	
Campylobacter jejuni (10)	0.3–2.5	2.5		
Candida albicans (2)	50		>50	
Candida glabrata (2)	50		50 to >50	
Candida parapsilosis (2)	50		50 to >50	
Enterococcus faecalis (6)	25		50	
Escherichia coli (4)	25–50		25–50	
Helicobacter pylori (4)	0.6–1.2			
Klebsiella pneumoniae (3)	50		50	
Kocuria rhizophila (1)	12.5		50	
Lactobacillus acidophilus (1)	6.2		12.5	
Lactobacillus casei (3)	12.5–25		12.5–25	
Lactobacillus spp. (13)	12.5–25	25	25	25
Listeria innocua (1)	12.5		12.5	
Listeria monocytogenes (8)	25		25–50	
Micrococcus luteus (1)	6.2		25	
Pseudomonas aeruginosa (4)	25–50		50	
Salmonella enterica subsp. *enterica* (1)	25		25	
Serratia marcescens (3)	25–50		25–50	
MSSA (12)	0.8–6.2	6.2	0.8–6.2	6.2
MRSA (17)	0.8–12.5	12.5	0.8–12.5	12.5
Staphylococcus capitis (2)	3.1		3.1	
Staphylococcus epidermidis (4)	1.6–3.1		1.6–3.1	
Staphylococcus hominis (2)	6.2		6.2	
Staphylococcus xylosus (2)	6.2–25		6.2–25	
Streptococcus pyogenes (10)	3.1–25	25	6.2–50	50

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MSSA, meticillin-susceptible *Staphylococcus aureus*; MRSA, meticillin-resistant *S. aureus*.

biophenols. One report describing the activity of an extract from olive leaves showed that *B. subtilis* was the least susceptible organism, whereas *E. coli*, *P. aeruginosa* and *K. pneumoniae* were the most susceptible, followed by *S. aureus* [5]. Another report also found that *B. subtilis* was the least susceptible organism, followed by *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *E. coli*. The most susceptible organisms were *Candida albicans* and *Bacillus cereus* [10]. A similar trend was seen in the current study whereby *B. cereus* was also more susceptible to OLE than *B. subtilis*, however it was not the most susceptible organism. Whilst there were some similar observations or trends between the current and previous studies, the majority of results differed. One possible explanation for this is that in both previous studies the OLE was extracted from powdered leaves, with the additional steps of either boiling [10] or autoclaving [5], whereas the extract used in the current study was obtained from fresh leaves.

In conclusion, the susceptibility data obtained in this study do not provide strong support for the claim that OLE is an effective therapy for a broad range of infectious diseases. However, the specific antimicrobial activity found against *H. pylori* and *C. jejuni* is of great interest and requires further investigation. Data for these organisms suggest that OLE, or other olive products such as olives or olive oil, may have a potential role in beneficially altering the composition of the gastric flora by selectively reducing levels of these two organisms. A similar suggestion has been made previously after the biophenols found in olive oil were shown to be active against *H. pylori* in vitro [11]. Furthermore, it is possible that after ingestion and metabolism of OLE, the biophenol metabolites may have more antimicrobial activity than the unmetabolised components [12], meaning that in vitro tests may underestimate total activity.

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