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Isolation and antimicrobial activity of a lanosol derivative from *Osmundaria serrata* (Rhodophyta) and a visual exploration of its biofilm covering

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Abstract

A biologically active compound, lanosol ethyl ether (LEE), was isolated from *Osmundaria serrata* and its antimicrobial activity was determined against various terrestrial bacteria and fungi, and marine bacteria isolated from the surface of the seaweed. This is the first report of the presence of a lanosol derivative in *O. serrata*. The mean bacteriostatic and fungistatic activity of LEE was 0.27±0.07 mg ml^{-1} and the mean bactericidal and fungicidal activity was 0.69±0.15 mg ml^{-1}. These values are comparable to the estimated concentration of LEE in the whole plant of 0.20 mg ml^{-1}. Extracts of *O. serrata* containing LEE also caused deformities in some bacteria that were tested. Biofilms cause the fouling of surfaces in the marine environment. The use of paints containing highly toxic metals to control their growth are ecologically harmful. Alternatives are required. The biofilm growing on the surface of this red seaweed was studied by light and scanning electron microscopy. A diverse biofilm was seen on *O. serrata* with bacteria even seen close to the meristematic tips of the plant. Further work is required to determine whether the concentration of the active compound in the tips is bactericidal.

Keywords: Antimicrobial; *Osmundaria serrata*; Lanosol; Biofilm

1. Introduction

The red seaweed *Osmundaria serrata* (Suhr) R.E. Norris (Figs. 1 and 2) grows attached to rocks and has been found along the east coast of South Africa and the Maldive Islands (Norris, 1991). It presumably also occurs in between these localities, but is probably restricted to the warm tropical and subtropical waters of the Indian Ocean since it does not occur in the colder waters off the western and southern coasts of South Africa. Macroalgae living on rocky shores have to tolerate high sheer forces from wave action and abrasive sand particles in the water. They also have to contend with herbivores and potentially virulent microbiota and commonly use physical and chemical means of protection. The crude extract of *O. serrata* has good antimicrobial activity indicating the presence of an active compound (Barreto, 2003). The antimicrobial activity of the active compound isolated from *O. serrata* was examined by growth studies using marine bacteria isolated from its surface and terrestrial bacteria and fungi.

Extracts from this seaweed have been found to induce deformities in fungi, where the culture characteristics of *Verticillium* sp. and *Rhizoctonia solani* were altered with prolific chlamydospore production observed in the latter (Barreto, 1995). Transmission electron microscopy (TEM) was used to examine the effects of extracts of *O. serrata* on the morphology of certain bacteria.

The fouling of all submerged surfaces in the marine environment develops from the initial adsorption of macromolecules and bacteria. Other microorganisms and macroorganisms may then succeed in colonising the surface to eventually form a thick covering that has negative economic impacts such as increased fuel consumption for ships and maintenance costs for harbours (Azis et al., 2003). At present toxic paints containing metals such as copper, tin and zinc are used to protect marine surfaces from fouling. Their accumulation along shipping routes and harbours is causing detrimental

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Fig. 1. Drawing of *O. serrata*—whole specimen and detail.

ecological effects, for example, mass mortality of bottlenose dolphins (Ponasik et al., 1998; Hellio et al., 2001; Katranitsas et al., 2003; Islam and Tanaka, 2004). These cytotoxic compounds also pose a direct risk to humans through contamination of the marine food web (De Sousa et al., 1998; Horiguchi et al., 2002; Bhosle et al., 2004). Organic biocides and "biocide-free" self-polishing antifouling paints are being used as alternatives, but also contaminate the aquatic environment with concerns for potential ecological problems due to their toxicity (Konstantinou and Albanis, 2004; Yebra et al., 2004; Löschau and Krätke, 2005). Bacteria in the biofilm of seaweeds produce antibiotic compounds that prevent fouling organisms from settling and growing (De Nys and Steinberg, 2002). The protective biofilm layer of *O. serrata* was explored using stereo-light microscopy and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Isolation and identification of active compound from O. serrata

Seaweed material was collected from Palm (30°59′30″S, 30°16′30″E) and Trafalgar Beaches (30°57′30″S, 30°18′00″E; both 30 30 CD), KwaZulu-Natal, South Africa in June 2002. The samples were cleaned of epiphytes, air-dried in the shade and stored in plastic bags for 3 days prior to being extracted.

In the isolation of the active compound from *O. serrata*, 1 kg of air-dried material was homogenised with 5 L ethanol and extracted for 1 week on a shaker at 21 ± 1 °C. The extract was filtered through Whatman No. 1 filter paper using a Buchner funnel. It was then dried under reduced pressure at 40 °C and stored under nitrogen at 0 °C. The seaweed material was re-extracted with 5 L ethanol-ethyl acetate (1:1) for another week, and again with 5 L ethyl acetate for another week. The combined extracts were dried and stored as before. The ethyl acetate soluble fraction was separated in a column (5 cm × 75 cm) packed with dry silica gel 60 (Merck) and eluted with gradient steps of hexane and ethyl acetate (1.45 L hexane, 1.15 L 8:2, 0.70 L 7:3, 0.90 L 1:1, 0.85 L 3:7 and 1.50 L ethyl acetate). The activity of the fractions was determined by bio-autography on silica gel thin-layer chromatography (TLC) plates and developed with hexane–ethyl acetate (1:1). Once dried, the TLC plates were sprayed with a spore suspension of *Alternaria alternata* (obtained from the Plant Pathology Department, University of Pretoria) in malt extract broth. The plates were then incubated at 25 °C for 3 days. The active fraction (light spots in a dark background of spores) was separated further in another silica gel column eluted with hexane–ethyl acetate (9:1). The active fractions were combined and repeatedly separated in a Sephadex LH-20 column eluted with ethanol to give pure compound.

The proton, carbon and two-dimensional NMR data for the purified compound in $CDCl_3$ was obtained at 300 MHz for the proton and 75 MHz for the carbon spectra.

2.2. Antimicrobial activity of active compound from O. serrata

The first column in Table 1 shows the species of bacteria and fungi that were used in the bioassays. Marine bacteria isolated from the surface of *O. serrata* were used in the bioassays, as well as terrestrial bacteria and fungi of medical and/or economic importance. The bacteria were grown for 24 h at 21 ± 2 °C prior to

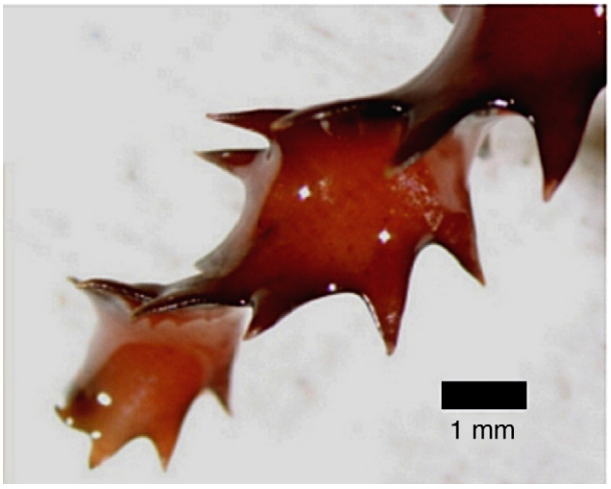

Fig. 2. Close-up view of a young portion of the thallus of *O. serrata* showing the serrated edges from which the species derives it name.