



Effect of *Ocimum tenuiflorum* Leaf Extract against Infective Endocarditis

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ABSTRACT

The ever increasing resistance of pathogens to antibiotics as well as the undesirable side effects of certain antimicrobial agents has necessitated the discovery of novel natural products. The ethanolic extract of *O. tenuiflorum* was investigated against *S. aureus* isolate which is a major pathogen causing infective endocarditis. The total phenolic content in *O. tenuiflorum* was found to be 1289 µg gallic acid equivalent/g and HPLC profile revealed the presence of catechin, Caffeic acid and p-coumaric acid. The antibacterial profile of *O. tenuiflorum* against *S. aureus* isolate from infective endocarditis patients exhibited significant inhibition at the MIC of 50 µg/ml and the standard antibiotic Vancomycin showed inhibition at 40 ng/ml. Percent inhibition of viable growth was found to be >95% and the scanning electron micrographs (SEM) revealed the disruption of the membrane of *S. aureus* treated with *O. tenuiflorum* extract. The antimicrobial activity correlates with phenolic content of the extract. These results demonstrate the potency of *O. tenuiflorum*, could serve as a new source of antimicrobials with potential applications and related health benefits.

Keywords: Infective Endocarditis; *O. tenuiflorum*; Antimicrobial; HPLC; SEM.

Introduction

Bacterial endocarditis is a disease characterized by inflammation or infection of the inner surface of the heart. Infective endocarditis is a disease affecting about 10,000 to 20,000 persons in the United States each year [1]. Endocarditis occurs when bacteria enter the bloodstream and attach to damaged portion of the inner lining of the heart or abnormal heart valves. Not all bacteria entering the bloodstream are capable of causing endocarditis. Only those bacteria that are able to stick to the surface lining of the heart and to abnormal valves tend to cause endocarditis. Endocarditis most often occurs in people with pre-existing heart disease and less commonly in people with normal heart [2]. Bacteria that normally live on the skin, the lining of the mouth, or the lining of the intestinal tract enter the bloodstream through small

cuts, abrasions, or breakdowns. The presenting signs and symptoms of infective endocarditis are highly variable, and the severity of illness ranges from mild to severe. Fever is almost always a symptom, other symptoms are loss of appetite, unexplained weight loss, new rashes (both painful and painless), headache, backache, joint pain, confusion, shortness of breath, or sudden weakness in the face or limbs suggestive of a stroke [3]. Untreated, most patients with infective endocarditis will die. The infection can lead to damage of the heart valve(s) that in turn causes severe leaking of blood back through the valve(s) and an inability of the heart to efficiently pump blood to the body, this in turn may lead to congestive heart failure [4].

Since, infective endocarditis can have serious consequences, it is important to try to prevent the



development of the disease. Treatment of endocarditis requires (a) intensive antimicrobial prophylaxis, sometimes for two, but often for four to six weeks. The type of antibiotic and the length of therapy depend on the results of the blood cultures, which identify the species of infecting bacteria and its sensitivity to specific antibiotics, (b) Surgical removal of the valve is necessary in patients who fail to clear micro-organisms from their blood in response to antibiotic therapy, or in patients who develop cardiac failure resulting from destruction of a valve by infection. Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led to investigate the antimicrobials from medicinal plants. Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is very important [5]. In the last few decades there has been an exponential growth in the field of herbal medicine. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases [6]. Hence, the present study aimed to investigate the effect of *O. tenuiflorum* (Lamiaceae) leaf extract against one of the etiological agent of infective endocarditis i.e., *S. aureus* and proving the mechanism of action by SEM and major constituents present in the extract by HPLC.

Materials and Methods

Plant Material and Extraction

The leaves of *Ocimum tenuiflorum* was collected in and around Mysore and authenticated from Department of Studies in Botany, University of Mysore, Mysore. Plant material was air dried in the dark and ground to a powder. Five grams of the plant material was extracted in ethanol in Soxhlet extractor and evaporated in rotary evaporator. The residue was stored at 4°C for further use.

Determination of Total Phenolic Content

The total phenolic content of the herbal extracts were determined colorimetrically using the Folin-Ciocalteu method [7]. A sample aliquot of 100 µl was added to

900 µl of water, 5 ml of 0.2 N Folin-Ciocalteu reagent and 4 ml of saturated sodium carbonate solution (100 g/l). The absorbance was measured at 765 nm after incubation for 2 h at room temperature. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram sample.

High performance liquid chromatography

Identification of polyphenols in the extract was carried out by RP-HPLC (LC-10A liquid chromatography LC: Shimadzu, Japan) in C18 column using diode array UV detector at 200-600 nm. The mobile phase - Acetonitrile: Water (70:30, v/v) with 1% of formic acid (v/v). Identification of the phenolic compounds was carried out by comparing their retention times with known standards (catechin, caffeic acid and p-coumaric acid).

Determination of MICs

The MICs of *O. tenuiflorum* extract and vancomycin antibiotic against *S. aureus* were determined by broth dilution assay with an inoculum density of $\sim 5 \times 10^5$ cfu/ml [8].

Determination of *S. aureus* cells for loss of viability

A sterile 100 ml flask containing 20 ml of 20 mM phosphate buffer pH 7.0 with 0.625% (w/v) BSA and 1% (v/v) DMSO. A second flask containing 20 ml of 50 µg/ml *O. tenuiflorum* extract was then prepared. Pure DMSO was employed as solvent control and this was diluted to a final concentration of 1% (v/v) with 20 mM sodium phosphate buffer containing 0.625% (w/v) BSA. The two flasks were pre-warmed to 37 °C by placing them in an orbital incubator at 100 rpm. These were then inoculated with $\sim 5 \times 10^7$ cfu/ml of *S. aureus*. After 0, 1, 2, 3, 4, 6, 8 and 12 h incubation, 0.1 ml samples were taken and viable counts were taken [9].

Examination of morphology of *S. aureus* cells treated with *O. tenuiflorum* extract under SEM

SEM was done according to [10] with several modifications. Briefly, 200 µl of 5,000-fold diluted ($\sim 5 \times 10^6$ cfu/ml) bacterial suspension were evenly plated onto the membranes and the dishes were incubated upright for 24 h at 37 °C. Membranes with the *S. aureus* cells were treated with 50 µg/ml of *O.*

tenuiflorum extract for 1 h. Then the membrane was rinsed with phosphate buffer saline for 15-30 s and fixed for 12 h in 50 mM phosphate buffer, containing 6.25% (w/v) glutaraldehyde. Samples were dehydrated with acetone, subjected to critical point drying, coated for 300 sec with gold palladium and inspected using a Zeiss DSM 962 SEM (Carl Zeiss, Oberkochen, Germany). Images were captured at low and high magnifying cation to show details of 3D-architecture.

Statistical analysis

All data are expressed as mean \pm SD. Statistical analysis was performed using one way ANOVA. Data was computed for statistical analysis by using SPSS statistical software. Statistical significance value was set at $p < 0.05$.

Results & Discussion

In the present investigation, the bioactivity studies of *O. tenuiflorum* extract was investigated. The results include, total phenolic content, presence of active

constituents in ethanolic extract, MIC and bactericidal activity of *O. tenuiflorum* extract. Previously, the presence of phenolic compounds in herbal extracts has been reported [7, 11]. The presence of total phenolic content in *O. tenuiflorum* extract was 1289 μg GAE/g. However, antimicrobial activity of plant extract is often associated with the phenolic compounds present in them. HPLC chromatograms showed the presence of catechin, caffeic acid and p-coumaric acid in sapodilla peel (Fig.1). The selection of these standards is due to their medicinal properties; Catechin mainly reduces atherosclerotic plaques [12], Caffeic acid suppresses acute immune and inflammatory response [13]. p- Coumaric acid has antioxidant properties and is believed to reduce the risk of stomach cancer by reducing the formation of carcinogenic nitrosamines [14]. The *O. tenuiflorum* extract contained appreciable quantities of catechin, caffeic acid and p-coumaric acid ($35 \pm 0.11 \mu\text{g/ml}$; t_R 2.7 min, $12.3 \pm 0.7 \mu\text{g/ml}$; t_R 3.12 min, and $5.72 \pm 0.12 \mu\text{g/ml}$; t_R 3.38 min respectively).

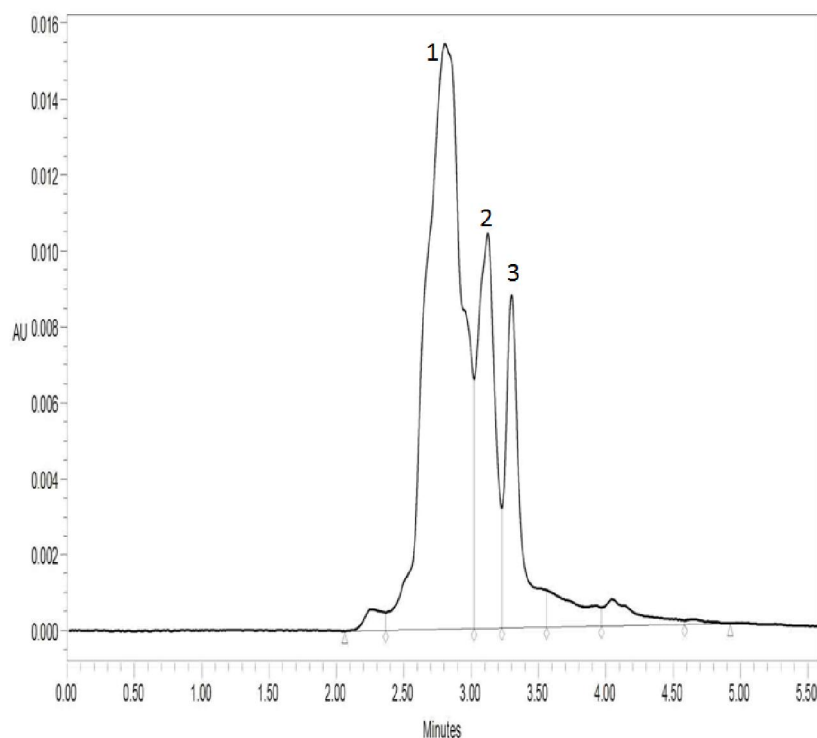


Fig 1. HPLC chromatograms of *O. tenuiflorum* extract. Peak 1: Catechin, Peak 2: Caffeic acid, Peak 3: p-Coumaric acid.

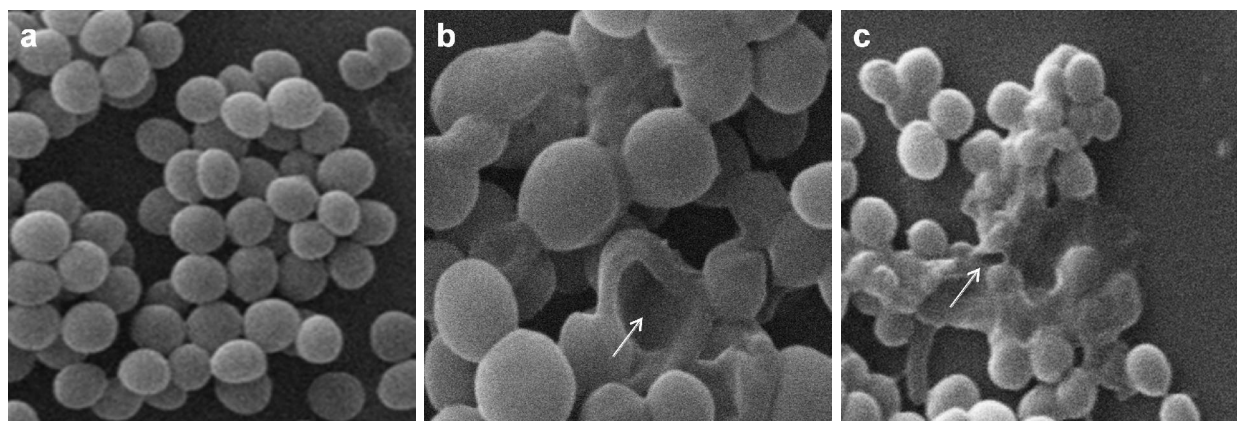


Fig 2. Scanning Electron micrograph showing the morphology of *S. aureus* (a) Control cells, (b) *O. tenuiflorum* extract treated cells and (c) Vancomycin treated.

S. aureus was sensitive to *O. tenuiflorum* at MIC 50 $\mu\text{g/ml}$ to that of MICs of vancomycin against *S. aureus* are 40 ng/ml. Cells suspended in 50 $\mu\text{g/ml}$ *O. tenuiflorum* extract showed >95% inhibition of viable growth compared to control cells. *O. tenuiflorum* extract inhibited/impaired the growth of *S. aureus* cells and the morphology was observed under SEM in the presence and absence of *O. tenuiflorum* extract. However, the addition of *O. tenuiflorum* extract at 50 $\mu\text{g/ml}$ on *S. aureus* was found to show mucilaginous mass of cells and also the disruption of the cell membrane which could lead to the impairment in cell division and chromosome replication (Fig.2b). However, vancomycin showed the disruption of the cell membrane (Fig. 2c) when compared to control cells remained stable (Fig. 2a). It is evident from the literature that the presence of Protein A is anchored to staphylococcal peptidoglycan pentaglycine bridges (chains of five glycine residues) by the transpeptidase sortase A. In fact, studies involving mutation of genes coding for protein A resulted in a lowered virulence of *S. aureus* as measured by survival in blood, which has led to speculation that protein A-contributed virulence requires binding of antibody Fc regions [15]. Our results emphasise on the disruption of the membrane of *S. aureus* which could be speculated that protein A is inhibited, which is major determinant of *S. aureus* virulence and hence bringing down the virulence of *S. aureus* and its survival.

The observation of membrane disruption in treated cells is very interesting and represents a useful lead worthy of further investigation. *O. tenuiflorum* extract may be damaging the cell membrane via Protein A inhibition lysis of *S. aureus* cells which is very much essential for preventing infective endocarditis. However, new classes of antimicrobial drug are required herbal principles represent a novel set of leads. Hence *O. tenuiflorum* extract could be attributed as a potential tool for the prevention of *S. aureus* related infective endocarditis and other related diseases.

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