

## ANTI-BIOFILM EFFICACY OF *PLECTRANTHUS AMBOINICUS* AGAINST *STREPTOCOCCUS PYOGENES* ISOLATED FROM PHARYNGITIS PATIENTS

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### ABSTRACT

**Objective:** The objective of the study was to evaluate the anti-biofilm efficacy of Indian medicinal plant *Plectranthus amboinicus* extracts against the biofilm forming *Streptococcus pyogenes* isolated from pharyngitis patients.

**Methods:** The plant extracts (methanol and ethyl acetate) were screened for their preliminary phytochemical components. The solvent extract with higher phytochemical yield was subjected to quantitative analysis using the Gas Chromatography-Mass Spectrometry (GC-MS) technique. *In vitro* analysis of the anti-biofilm study was performed using the Minimal Inhibitory Concentration (MIC) assay, biofilm inhibitory concentration assay, growth curve analysis, anti-bacterial activity, and light microscopy analysis.

**Results:** The methanol extract showed the highest phytochemical content. GC-MS analysis of the methanol extract showed a total of thirty two phyto compounds among which most of the compounds were medicinally important. MIC assay showed that the inhibition of test pathogens was at an average concentration of 2 mg/ml. The agar well diffusion method elucidated that at sub-MIC the methanol and ethyl acetate extracts did not inhibit the growth of the test pathogen. Growth curve analysis was carried out at the concentration sub-MIC, in which the solvent extracts did not show any significant inhibition on the planktonic cells, whereas the biofilms of the test pathogens were significantly reduced and were dose dependent at sub-MIC levels as confirmed by the light microscopic analysis.

**Conclusion:** These preliminary results indicated that the methanol extract of *P. amboinicus* leaves consisted of pharmacologically active components and could be used as an anti-biofilm agent at minimal concentrations thereby successful preventing the formation of biofilms.

**Keywords:** *Plectranthus amboinicus*, GC-MS, Anti-biofilm activity, Light microscopy, *S. pyogenes*.

### INTRODUCTION

The discovery of antibiotics and their application as chemotherapeutic agents emphasized that frequent administration would lead to the eradication of infectious diseases [1]. With the increase in the global emergence of multi-drug resistant bacterial strains, the effectiveness of anti-bacterial drugs has become limited leading to the failure in the treatment of infections [2]. Research has shown that majority of the bacterial cells form biofilm [3]. Biofilm formation protects the bacterial cells from environmental stress and antibiotic treatment by which they become more resistant. *Streptococcus pyogenes* is a major upper respiratory tract pathogen which causes bacterial pharyngitis that leads to serious complications and it is associated with extensive human morbidity worldwide [4,5]. The ability of this bacterium to form biofilms is one of the virulence-promoting factors as the bacteria are protected from the host immune system and antibiotics administered during the treatment period [6]. Biologically active compounds derived from herbal plants have always been a thrust area for the researchers working on infectious diseases and their control [7]. In India, a wide variety of aromatic plants are widely employed in traditional medicine in the treatment of infectious diseases as well as to extend the shelf life of foods [8,9].

The genus, *Plectranthus* consists of more than 300 species of plants and belongs to the family of Lamiaceae. Plants belonging to this family have rich ethnobotanical diversity with unique medicinal properties. One such medicinal plant in this genus is *Plectranthus amboinicus* [Lour.] Spreng, commonly known as Indian borage and it is widely used in the traditional medical systems of India [10]. The plant is a large, succulent, aromatic, perennial herb distributed throughout India and Sri Lanka [11]. *P. amboinicus* is medicinally used to treat urolithiasis, epilepsy, tumors and mutagens, neurological disorders, viral and fungal infections [12].

A decoction of its leaves are used to treat chronic cough and asthma and also used as an anti-spasmodic for, stomach ache, and for the treatment of a headache, fever, epilepsy, and dyspepsia [13]. It is also used in the treatment of skin ulcerations and urinary diseases, as well as to alleviate inflammation, kidney troubles and in conditions of congestive heart failure [14,15]. Although many reports have substantiated the various medicinal properties of *P. amboinicus*, the studies on the anti-biofilm properties of this plant are at a naïve stage. Moreover, based on the anti-bacterial efficacy of these plants against various bacterial pathogens, we hypothesize that the solvent extract would also aid in controlling the biofilm formation of *S. pyogenes*, which is one of the upcoming virulence factors for the pathogen. The present study is focused on the anti-biofilm properties of *P. amboinicus* solvent extracts against the biofilm forming Gram-positive pathogen *S. pyogenes*.

### MATERIALS AND METHODS

#### Collection of plant materials

*P. amboinicus* leaves were collected from area surrounding Coimbatore, Tamil Nadu. Specimens of *P. amboinicus* (Voucher No: 1113) have been authenticated as *P. amboinicus* [Lour.] Spreng. and deposited in the Botanical Survey of India, Southern Circle, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

#### Solvent extraction

The leaves were washed, shade dried, and powdered. About 25 g of the each dried plant powder was soaked in 100 ml of methanol and ethyl acetate (1:4) for 7 days with periodic soaking and then filtered using Whatman filter paper No. 1. The filtrate was then dried at 55 °C for 1 h using rotary vacuum evaporator (Buchi Type, India) and the yield percentage yield was calculated. The dried *P. amboinicus* methanol (PAM) and ethyl acetate (PAEA) extracts were then aliquoted using