

Phytochemical Investigation, Leaf Structure and Antimicrobial Screening of *Pistacia lentiscus* against Multi-Drug Resistant Bacteria

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Abstract : Introduction: *Pistacia lentiscus* L. (well known as Mastic tree) is an evergreen sclerophyllous shrub that extensively thrives in the eastern Mediterranean area yet only the trees cultivated in the southern region of the Greek island Chios produces mastic resin. Different parts of *P. lentiscus* L. var. chia have been used in folk medicine for various purposes, such as tonic, aphrodisiac, antiseptic, antihypertensive and management of dental, gastrointestinal, liver, urinary, and respiratory tract disorders. Several studies have focused on the antibacterial activity of its resin (gum) and its essential oil. However, there is no study combining anatomy of the plant organs, phytochemical profile, and antibacterial screening of the plant. In our attempt to discover novel bioactive metabolites from the mastic tree, we screened its antibacterial activity not only against ATCC strains but also against clinical, resistant strains. Materials-methods: Leaves were investigated using Transmission (TEM) and Scanning Electron Microscopy (SEM). Histochemical tests were performed on fresh and fixed tissue. Extracts prepared from dried, powdered leaves using 3 different solvents (DCM, MeOH and H₂O) the waste water obtained after a hydrodistillation process for essential oil production were screened for their phytochemical content and antibacterial activity. Metabolite profiling of polar and non-polar extracts was recorded by GC-MS and LC-HRMS techniques and analyzed using in-house and commercial libraries. The antibacterial screening was performed against *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 and against clinical, resistant strains Methicillin-resistant *S. aureus* (MRSA), Carbapenem-Resistant Metallo- β -Lactamase (carbapenemase) *P. aeruginosa* (VIM), *Klebsiella pneumoniae* carbapenemases (KPCs) and *Acinetobacter baumannii* resistant strains. The antibacterial activity was tested by the Kirby Bauer and the Agar Well Diffusion method. The zone of inhibition (ZI) of each extract was measured and compared with those of common antibiotics. Results: Leaf is compact with inosclereids and numerous idioblasts containing a globular, spiny crystal. The major nerves of the leaf contain a resin duct. Mesophyll cells showed accumulation of osmiophilic metabolites. Histochemical treatments defined secondary metabolites in subcellular localization. The phytochemical investigation revealed the presence of a large number of secondary metabolites, belonging to different chemical groups, such as terpenoids, phenolic compounds (mainly myricetin, kaempferol and quercetin glycosides), phenolic, and fatty acids. Among the extracts, the hydrodistillation wastewater achieved the best results against most of the bacteria tested. MRSA, VIM and *A. baumannii* were inhibited. Conclusion: Extracts from plants have recently been of great interest with respect to their antimicrobial activity. Their use emerged from a growing tendency to replace synthetic antimicrobial agents with natural ones. Leaves of *P. lentiscus* L. var. chia showed a high antimicrobial activity even against drug - resistant bacteria. Future prospects concern the better understanding of mode of action of the antibacterial activity, the isolation of the most bioactive constituents and the clarification if the activity is related to a single compound or to the synergistic effect of several ones.

Keywords : antibacterial screening, leaf anatomy, phytochemical profile, *Pistacia lentiscus* var. chia

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