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# *In vitro* Biological Activity of *Liquidambar orientalis* Mill against Pathogen and Marine Biofilm Microorganisms

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**The balsam "liquid storax" of *Liquidambar orientalis* Mill. that (Sigla) is an endemic tree species for Eastern Mediterranean, was obtained from Günnücek (Marmaris). It was extracted with n-hexane, $CH_2Cl_2$ and MeOH respectively. The n-hexane, $CH_2Cl_2$, MeOH extracts were tested at concentrations of 10.0%, 1.0%, 0.4%, 0.2% and 0.1% against two different groups of bacteria: Pathogens and marine biofilm bacteria. *Klebsiella pneumonia, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeroginosa, Bacillus cereus, Enterococcus faecalis and Candida albicans* were used as pathogen microorganisms whereas, *Pseudoalteromonas marina P. haloplanktis, Alteromonas alvinella, Alteromonas genoviensis Vibrio splendidus, Exiguobacterium homiense, Vibrio lentus* were used as marine biofilm bacteria. The antimicrobial test results showed that the MeOH extract of storax has more potent antimicrobial activity than $CH_2Cl_2$ and n-Hexane extracts. Inhibitory activities of *L. orientalis* resin extracts were high against biofilm bacteria, while they were found to be low against pathogenic microorganisms tested.**

**Key words:** *Liquidambar orientalis* Mill., Medicinal plant, Pathogen and biofilm bacteria.

Since the finding of antimicrobial activities in many species of folk medicinal plants from the world and isolation of some active compounds form them [1]. *Liquidambar orientalis* Mill. (Sigla) is an endemic tree species for Eastern Mediterranean, of Boreal-Tertiary origin and closely related to *L. styraciflua* from Eastern North America. It differs from the latter in its leaves being nearly always glabrous beneath (instead of constantly having a thick woolly pad of hairs in the axils of the main veins), and usually lobulate lobes. The Turkish *Liquidambar* forms a smaller, rounded tree than the American species which has a tall, columnar-pyramidal crown. *L. orientalis* is a medicinal plant. The balsam, "liquid storax" is produced by wounding the bark; 62 tons were exported to Europe and U.S.A, in 1960 [2].

The resin produced by injuring *L. orientalis* is also known as Levant Storax (Sigla Oil, Gunluk Oilin Turkey) [3]. It is a good antiseptic and is also used as a topical parasiticide, expectorant and for the treatment of some skin diseases [4]. It has a characteristic bitter taste and odour. The balsam has a wide application in the perfume industry as a fixative of the aromatic substances in the production of perfumes. It has been also used in other branches of cosmetics [4-6]. To the best our knowledge, there is limited number of reports on antimicrobial activities of the genus *Liquidambar* [5-8].

Marine biofilms have negative effects on human activities in many ways, including: biofouling, energy waste, and heat transfer resistance, requirement for excess equipment capacity, decreased life of equipment, quality

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control problems, and safety problems[9-10]. The objectives of this study were to access the antimicrobial activities of crude n-hexane, $CH_2Cl_2$ and methanol storax extracts against pathogens and arine biofilm bacteria.

## MATERIALS AND METHODS

### Plant material and extraction

The storax sample obtained from Günnücek. The storax (250g) was extracted with n-Hexane (2x L) at room temperature. After filtration of extract, hexane phase was evaporated to dryness under reduced pressure and controlled temperature (40-50 $^0$C) in a rotary evaporator six gram. Hexane extract was obtained then, extraction was carried out by $CH_2Cl_2$ (2xL). The $CH_2Cl_2$ extract was evaporated by under the vacuum seven gram. $CH_2Cl_2$ extract was obtained as a gummy mixture. The residue of storax was extracted with MeOH finally, on evaporation afforded a gummy mixture (5.69g).

### Test microorganisms

The n-hexane, $CH_2Cl_2$, MeOH extracts were tested against two different groups of microorganisms: Pathogens (bacteria and yeast strain) and biofilm bacteria. *Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 29998, *Salmonella typhimurium* CCM 583, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeroginosa* ATCC 27853, *Bacillus cereus* ATCC 7064, *Enterococcus faecalis* ATCC 8043 and *Candida albicans* ATCC 10239 were used as pathogen microorganisms whereas *Pseudoalteromonas marina* FJ200642, *P. haloplanktis* FJ040186, *P. elyakovii* FJ200650, *P. porphyrae* FJ200651, *P. agarivorans* FJ040188, *Alteromonas alvinella* FJ200645, *A. genoviensis* FJ200641, *Vibrio splendidus* FJ200647, *V. lentus* FJ200649, *Exiguobacterium homiense* FJ200653 were used as biofilm forming seawater bacteria [11].

### Antimicrobial analysis:

The antimicrobial activity of all extracts was determined by using the paper disk diffusion method. The pathogen bacteria strains were inoculated on Nutrient Broth (Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 37 °C, while the yeast strain was inoculated on Malt Extract Broth (Oxoid) and incubated for 48 h at 28 $^o$C and biofilm bacteria were inoculated on Zobell Agar (Oxoid) and incubated for 24 h at 26 $^o$C. The counts of bacteria strains and yeast strain were adjusted to yield approximately $10^7$- $10^8$ cfu ml$^{-1}$ and $10^5$- $10^6$ cfu ml$^{-1}$, respectively, using the Standard McFarland counting method. The extracts (all extracts were filter-sterilized using a 0.22 µm membrane filter) were prepared at 10.0%, 1.0%, 0.4%, 0.2% and 0.1% concentrations in correspond solvent. The test organisms (100µl) were inoculated with a sterile swab on the surface of appropriate solid medium (Mueller Hinton Agar for pathogen bacteria, Zobell Agar for biofilm bacteria and Malt Extract Agar for yeast) in plates. The agar plates inoculated with the test organisms were incubated for 1 h before placing the extract impregnated paper disks on the plates [11-12]. Sterile paper disk of 6 mm diameter were impregnated these extracts (50 µl) [13]. The bacterial plates were incubated at 37 $^o$C and 26 $^o$C for 24 h, and the yeast plates were incubated at 28 $^o$C for 48 h. After incubation, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters [14]. All tests were performed under sterile conditions in duplicated and disks imbued with 50 µl of pure n-hexane, $CH_2Cl_2$, MeOH were used as a negative control.

## RESULTS AND DISCUSSION

In vitro antibacterial activity of *L. orientalis* resin extracts against different pathogenic microorganisms and biofilm bacteria with varied concentrations were shown in Table 1 and 2. The inhibition zones were varied related to different concentrations of *L. orientalis* extracts. Our results demonstrated remarkable that MeOH extract of storax showed more potent antimicrobial activity than $CH_2Cl_2$ and n-Hexane extracts.

In case of the MeOH extract of *L. orientalis*, the diameters of growth inhibition zones ranged from 8 to 12 mm, with the highest inhibition zone value exhibited moderate activity against the medically important *E. coli* (12 mm for all concentrations, except 0.1%). However, the diameters of growth inhibition zones ranged from 9 to 20 mm, with the highest inhibition zone values observed against the biofilm bacteria *A. alvinella* (20 mm for 10% concentration, *P. elyakovii* (20 mm for 10% concentration, and *E. homiense* (20 mm for 1 and 10% concentrations. As can be seen in

**Table 1**. Antibacterial activity against pathogens of the storax extracts

| Extracts | n-Hexane | | | | | $CH_2Cl_2$ | | | | | MeOH | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentrations | 10% | 1% | 0.4% | 0.2% | 0.1% | 10% | 1% | 0.4% | 0.2% | 0.1% | 10% | 1% | 0.4% | 0.2% | 0.1% |
| Microorganisms | | | | | | | | | | | | | | | |
| *Salmonella typhimurium* | - | - | - | - | - | 8±1.0 | 8±0.0 | - | - | - | 9±1.0 | 9±1.0 | 8±0.0 | - | - |
| *Staphylococcus aureus* | - | - | - | - | - | 10±0.0 | - | - | - | - | 10±0.0 | 10±1.0 | 10±1.0 | 10±0.0 | 10±0.0 |
| *Pseudomonas aeruginosa* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Escherichia coli* | - | - | - | - | - | - | - | - | - | - | 12±1.0 | 12±1.0 | 12±0.0 | 12±0.0 | 10±0.0 |
| *Enterococcus faecalis* | - | - | - | - | - | - | - | - | - | - | 10±1.0 | 10±1.0 | 10±1.0 | 10±1.0 | 10±0.0 |
| *Klebsiella pheumonia* | 8±1.0 | 8±1.0 | 8±1.0 | 8±0.0 | 8±0.0 | - | - | - | - | - | 10±0.0 | 10±0.0 | 10±0.0 | 10±0.0 | 8±0.0 |
| *Bacillus cereus* | 10±1.0 | 10±1.0 | 10±0.0 | 10±0.0 | 8±0.0 | - | - | - | - | - | 10±1.0 | 10±1.0 | 9±1.0 | 9±1.0 | 9±0.0 |
| *Candida albicans* | 8±1.0 | 8±0.0 | - | - | - | 8±1.0 | 8±1.0 | 7±1.0 | 7±0.0 | 7±0.0 | 10±1.0 | 10±1.0 | 10±1.0 | 10±1.0 | 8±0.0 |

(Mean values± SE)

**Table 2**. Antibacterial activity against biofilm bacteria of the storax extracts

| Extracts | n-Hexane | | | | | $CH_2Cl_2$ | | | | | MeOH | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentrations<br>Microorganisms | 10% | 1% | 0.4% | 0.2% | 0.1% | 10% | 1% | 0.4% | 0.2% | 0.1% | 10% | 1% | 0.4% | 0.2% | 0.1% |
| *Alteromonas alvinella* | 10±1.0 | 9±1.0 | 8±1.0 | 8±0.0 | 8±0.0 | 13±2.0 | 10±1.0 | 8±1.0 | 8±0.0 | - | 20±2.0 | 15±2.0 | 15±1.0 | 13±1.0 | 13±1.0 |
| *Vibrio lentus* | 10±0.0 | 10±0.0 | - | - | - | 10±0.0 | - | - | - | - | 14±1.0 | 14±1.0 | 13±1.0 | 13±1.0 | 12±0.0 |
| *Vibrio splendidus* | 11±2.0 | 7±1.0 | - | - | - | 8±1.0 | 7±1.0 | 7±1.0 | - | - | 14±1.0 | 14±1.0 | 13±1.0 | 13±1.0 | 13±1.0 |
| *Alteromonas genoviensis* | 12±1.0 | 8±1.0 | 8±0.0 | 8±0.0 | 8±0.0 | 13±2.0 | 10±2.0 | 10±0.0 | 8±0.0 | 8±0.0 | 15±2.0 | 14±0.0 | 14±0.0 | 14±0.0 | 13±0.0 |
| *Exiguobacterium homiense* | 11±2.0 | 11±2.0 | 6±1.0 | 6±1.0 | - | 20±2.0 | 13±2.0 | 10±2.0 | 8±1.0 | 6±0.0 | 20±1.0 | 20±1.0 | 18±1.0 | 12±0.0 | 12±0.0 |
| *Pseudoalteromonas agarivorans* | 9±1.0 | 9±0.0 | 9±0.0 | 9±0.0 | 9±0.0 | 8±1.0 | 7±1.0 | 7±1.0 | 7±1.0 | 7±0.0 | 15±2.0 | 14±1.0 | 13±1.0 | 13±1.0 | 13±1.0 |
| *Pseudoalteromonas elyakovii* | 10±0.0 | 9±0.0 | 8±1.0 | 8±0.0 | 8±0.0 | 10±2.0 | 10±1.0 | 8±0.0 | 8±0.0 | 8±0.0 | 20±2.0 | 19±1.0 | 19±1.0 | 17±1.0 | 17±1.0 |
| *Pseudoalteromonas porphyrae* | 11±2.0 | 9±1.0 | 8±1.0 | 8±1.0 | - | 8±1.0 | 8±1.0 | 8±0.0 | 7±0.0 | 7±0.0 | 13±1.0 | 13±1.0 | 12±0.0 | 12±0.0 | 9±.00 |
| *Pseudoalteromonas haloplanktis* | 10±0.0 | 8±0.0 | 8±0.0 | 8±0.0 | 8±0.0 | 8±0.0 | 8±0.0 | 8±0.0 | 7±0.0 | 7±0.0 | 14±2.0 | 14±2.0 | 13±1.0 | 13±1.0 | 11±1.0 |
| *Pseudoalteromonas marina* | 8±1.0 | 8±1.0 | 8±0.0 | 8±0.0 | 8±1.0 | 8±1.0 | 8±1.0 | 8±0.0 | 8±0.0 | 8±0.0 | 13±1.0 | 13±1.0 | 13±1.0 | 13±1.0 | 10±0.0 |

(Mean values± SE)

Table 1 methanol extract did not exhibit antimicrobial activity against *P. aeruginosa*.

Though, dichloromethane extract was found to have activity against *S. aureus* (10 mm for 10% concentration. Except for *S. aureus, S. typhimurium* and *C. albicans* the dichloromethane extract of *L. orientalis*, showed no antimicrobial activity against the other pathogenic microorganisms. For the biofilm bacteria, the dichloromethane extract, the diameters of growth inhibition zones ranged from 6 to 20 mm, with the highest inhibition zone value observed against biofilm bacterium *E. homiense* (20 mm for 10% concentration). All concentrations of hexane extracts were inactive against *S. typhimirium*, *S. aureus, P. aeroginosa, E. coli*, and *E. faecalis* while the extracts only had an inhibitory effect against *B. cereus* (10 mm), *K. pneumonia* (8 mm), and *C. albicans* (8 mm). However, hexane extract was exhibited highest activity against biofilm bacterium *A. genoviensis* (12 mm for 10% concentration). All microorganisms were completely unsusceptible to control disks imbued with solvents. In their study, Sagdic *et al.* [7] found that, the *L. orientalis* storax which was dissolved in ethanol and was tested at variable concentrations, had antibacterial activity (6-16mm) against many bacteria (*Bacillus cereus, B. brevis, B. subtilis, Enterobacter aerogenes, E. faecalis, Staphylococcus aureus, Klebsiella pneumonia*) for 10 % concentrations. In a study by Ahanjan *et al.* [15], it was determined that, extracts of leaves of *Parrotia persica* (deciduous tree of the family Hamamelidaceae) were evaluated for antibacterial activity against pathogen bacteria; methanol extract had significantly high degree of antibacterial activity. They suggested that methanol was the appropriate solvent for extraction of the antibacterial principle and was recommended for the large-scale extraction of the active principle.

In conclusion, concerning the antimicrobial activity of Liquidambar orientalis extracts against pathogens, the MeOH extract of storax showed more potent antimicrobial activity than $CH_2Cl_2$ and n-Hexane extracts. Inhibitory activities of *L. orientalis* extracts were high or moderate against biofilm bacteria, while they were found to be low against pathogenic microorganisms tested. Previous studies have been focused on the antibacterial activity of *L. orientalis* against pathogens. Therefore, it is difficult to compare the results of our study on *L. orientalis* and there are no reports on the anti-biofilm activity of *L. orientalis*. These results will provide a starting point for the investigations to exploit new marine paints additive and ingredient substances present in the extracts of the plant studied.

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