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Antimicrobial and Antibiofilm activities of Sea Buckthorn essential oil against E. coli, E. faecalis and St. mutans

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Abstract
Introduction: Sea buckthorn (Hippophae rhamnoides) is a plant found throughout Europe and Asia. It produces orange-yellow berries, which have been used over centuries as food, traditional medicine and skin treatment. Escherichia coli is a gram-negative bacillus known to be a part of normal intestinal flora but can also be the cause of intestinal and extra intestinal illness in humans. Enterococcus faecalis is a Gram-positive commensal member of the gut microbiota and is commonly detected in acute periodontitis. As a major etiological agent of human dental caries, Streptococcus mutans lives primarily in biofilms on the tooth surfaces, the so-called dental plaque. **Materials and Methods:** Subcultures were grown on MHA, MIC and Anti biofilm Activity were analyzed and subjected to elisa plate reading. **Results:** The zones of clearance were seen for all three species of bacteria only at the highest concentration of 40 µl. **Discussions:** Sea buckthorn has a variety of beneficial effects including anti-aging and antioxidant properties. **Conclusion:** Sea Buckthorn essential oil potentially inhibited the microbial growth and biofilm formation by E. coli, St. mutans and E. Faecalis.
Keywords: Sea buckthorn; Hippophae rhamnoides; Essential oil; Antimicrobial activity; Antibiofilm activity; Escherichia coli; Enterococcus faecalis; Streptococcus mutans; Dental plaque; Minimum inhibitory concentration (MIC); Muller Hinton Agar; Microtiter plate assay; Oral pathogens; Natural phytochemicals; Plant-derived antimicrobial agents.

Introduction

Sea buckthorn (*Elaeagnus rhamnoides* is a bush or small tree. It belongs to the *Elaeagnaceae* family, which is naturally distributed throughout Eurasia from the Baltic Sea and North Sea in the west to Central Asia in the east (1). Although a unique mixture of bioactive compounds is found throughout the plant, this is especially the case for the fruits, which are known as seaberry or Siberian pineapple (1,2). In vitro studies, and in vivo human and animal models, have found the juices, jams and oil derived from the fruits to have a range of beneficial anti-inflammatory, anti-cancer, antioxidant and anti-atherosclerotic effects; these have been attributed to the presence of phenolics, vitamins, minerals, amino acids, fatty acids and phytosterols(3,4). Plant oils are usually lipophilic or hydrophobic materials which can be extracted with nonpolar solvents such as hexane. The presence of highly nutritional and medicinal components in the oil from sea buckthorn fruits has prompted a growth of interest in its potential as a functional food product (5). Oil can be extracted from the seeds and the pulp (Zeb, 2006, Christaki, 2012): the mature seeds contain 8–20% oil, the dried fruit pulp (flesh and peel) about 20–25%, and the fruit residue contains about 15–20% oil after juice extraction (6). These oils have high concentrations of lipophilic constituents, predominantly unsaturated fatty acids (UFAs) in triglyceride form, phytosterols and vitamins A and E; these have a positive influence on human health, especially on the cardiovascular system (7). However, it is important to emphasize that the precise cardioprotective influence of the oil on the human organism remains unclear, and although some papers offer a glimpse into its effects, further animal studies are needed to better appreciate the true value of sea buckthorn as a source of medical and nutritional compounds. ***Escherichia coli*** is a paradigm for a versatile bacterial species which comprises harmless commensal as well as different pathogenic variants with the ability to either cause intestinal or extraintestinal diseases in humans and many animal hosts (8). Because of this broad spectrum of lifestyles and phenotypes, E. coli is a well-suited model organism to study bacterial evolution and adaptation to different growth conditions and niches. The geno- and phenotypic diversity, however, also hampers risk assessment and strain typing (9). A marked genome plasticity is the key to the great variability seen in this species. Acquisition of genetic information by horizontal gene transfer, gene loss as well as other genomic modifications, like DNA rearrangements and point mutations, can constantly alter the genome content and thus the fitness and competitiveness of individual variants in certain niches. Specific gene subsets and traits have been correlated with an increased potential of E. coli strains to cause intestinal or extraintestinal disease (10). Intestinal pathogenic E. coli strains can be reliably discriminated from non-pathogenic, commensal, or from extraintestinal E. coli pathogens based on genome content and phenotypic traits. ***Enterococcus faecalis*** – formerly classified as part of the group D *Streptococcus* system – is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans (11). Like other species in the genus *Enterococcus*, *E. faecalis* is found in healthy humans and can be used as a probiotic. The probiotic strains such as Symbioflor1 and EF-2001 are characterized by the lack of specific genes related to drug resistance and pathogenesis (12). As an opportunistic pathogen, *E. faecalis* can cause life-threatening infections, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contribute to its pathogenicity(13). *E. faecalis* has been frequently found in reinfected, root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases. Re-infected root canal-treated teeth are about nine times more likely to harbor *E. faecalis* than cases of primary infections. Endodontic treatment failure may occur due to different causes such as persistence of bacteria, root canals that are poorly cleaned and obturated, improper coronal seal (leakage), and untreated canals (missed canals) (14). The main reason for endodontic failure is the presence of some species of bacteria inside the root canal system such as Enterococcus (E.) faecalis. Those bacteria are more resistant to disinfection agents, causing a persistent intra-radicular or extra-radicular infection. (15)). In some diseases, a very important role is played by the ability of bacteria to form a multi-dimensional complex structure known as biofilm. The most common disease of the oral cavity, known as dental caries, is a top leader(16) . *Streptococcus mutans*, one of the many etiological factors of dental caries, is a microorganism which is able to acquire new properties allowing for the expression of pathogenicity determinants determining its virulence in specific environmental conditions (17). Through the mechanism of adhesion to a solid surface, S. mutans is capable of colonizing the oral cavity and also of forming bacterial biofilm. Additional properties enabling S. mutans to colonize the oral cavity include the ability to survive in an acidic environment and specific interaction with other microorganisms colonizing this ecosystem. (18)

Materials and Methods

Materials

Sea buckthorn (Hippophae rhamnoides) essential oil was procured from **Aramacks Pvt. Ltd., Delhi, India**. All microbiological media and reagents used in the study were of analytical grade. **Muller Hinton Agar (MHA)** was used for antimicrobial susceptibility testing. **Triphenyl tetrazolium chloride (TTC)** was used as an indicator for the determination of bacterial growth during minimum inhibitory concentration (MIC) analysis. Crystal violet dye was used for assessing biofilm formation in microtiter plates.

The bacterial strains used in the study included **Escherichia coli, Streptococcus mutans, and Enterococcus faecalis**, which represent clinically significant pathogens associated with intestinal infections and oral diseases such as dental caries and periodontitis.

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Preparation of Bacterial Cultures
Pure cultures of *E. coli*, *S. mutans*, and *E. faecalis* were obtained and maintained under standard microbiological conditions. Subcultures were prepared by inoculating the bacterial strains into appropriate nutrient media and incubating them under optimal growth conditions. Fresh bacterial suspensions were prepared prior to antimicrobial testing to ensure active growth and viability of the microorganisms.

Antimicrobial Activity (Zone of Inhibition Assay)
The antimicrobial activity of Sea buckthorn essential oil was evaluated using the **agar well diffusion method** on Muller Hinton Agar plates. Sterile MHA plates were inoculated with standardized bacterial suspensions using sterile cotton swabs to ensure uniform distribution of the microorganisms.

Wells were created in the agar medium using a sterile cork borer, and different concentrations of Sea buckthorn essential oil (**40 µl, 30 µl, and 20 µl**) were introduced into the wells for each bacterial strain. The plates were incubated at **37°C for 24 hours**. After incubation, the **zones of inhibition** surrounding the wells were measured in millimeters to determine the antimicrobial effectiveness of the essential oil against the tested microorganisms.

Determination of Minimum Inhibitory Concentration (MIC)
The **minimum inhibitory concentration (MIC)** of Sea buckthorn essential oil against the selected bacterial strains was determined using a **two-fold serial dilution method**. Serial dilutions of the essential oil were prepared in broth medium to obtain a range of concentrations. Each dilution was inoculated with the bacterial suspension and incubated for **48 hours** under appropriate conditions. Following incubation, **TTC solution** was added as a growth indicator. The MIC was determined as the lowest concentration of Sea buckthorn essential oil that prevented visible bacterial growth, indicated by the absence of color change after the addition of TTC.

Anti-Biofilm Activity Assay
The ability of Sea buckthorn essential oil to inhibit biofilm formation was evaluated using a **microtiter plate assay** combined with **crystal violet staining**. Bacterial cultures were inoculated into microtiter plates containing nutrient broth along with varying concentrations of the essential oil.

After incubation, the wells were gently washed to remove planktonic cells, and the remaining biofilm was stained using crystal violet. The stained biofilms were then quantified by measuring absorbance using an **ELISA plate analyzer at a wavelength of 525 nm**. The reduction in optical density values compared with control samples indicated the inhibitory effect of Sea buckthorn oil on bacterial biofilm formation.

Results
The antimicrobial and antibiofilm activities of Sea buckthorn essential oil were evaluated against **Escherichia coli, Enterococcus faecalis, and Streptococcus mutans**, which are important bacterial species associated with intestinal and oral infections.

Antimicrobial Activity
The agar diffusion assay demonstrated that Sea buckthorn essential oil exhibited **dose-dependent antimicrobial activity** against all tested bacterial strains. Clear zones of inhibition were observed around the wells containing the essential oil, indicating suppression of bacterial growth.

Among the tested concentrations, **the highest concentration of 40 µl produced visible zones of clearance for all three bacterial species**, indicating effective antimicrobial activity. Lower concentrations of 30 µl and 20 µl produced comparatively smaller or negligible zones of inhibition, suggesting that the antimicrobial effect of Sea buckthorn oil increases with concentration.

The observed antibacterial activity may be attributed to the presence of **bioactive compounds such as flavonoids, phenolic compounds, vitamins, and unsaturated fatty acids** present in Sea buckthorn oil, which can disrupt bacterial cell membranes and inhibit microbial metabolic processes.

Minimum Inhibitory Concentration (MIC)
The MIC analysis further confirmed the antimicrobial potential of Sea buckthorn essential oil. The results indicated that the essential oil inhibited bacterial growth at different minimum concentrations depending on the bacterial species tested.

The **lowest inhibitory concentration observed for *Escherichia coli* was 0.312% (v/v)**, indicating that this organism was relatively more sensitive to Sea buckthorn oil compared to the other bacterial strains.

For **Enterococcus faecalis**, the minimum inhibitory concentration was determined to be **0.625% (v/v)**, demonstrating moderate sensitivity to the essential oil.In the case of **Streptococcus mutans**, which is a primary causative agent of dental caries, bacterial growth inhibition was observed at a concentration of **1.5% (v/v)**. This indicates that *S. mutans* exhibited comparatively higher resistance to the antimicrobial action of Sea buckthorn oil.

Anti-Biofilm Activity
The antibiofilm assay demonstrated that Sea buckthorn essential oil was capable of **inhibiting biofilm formation by *Escherichia coli*** at relatively low concentrations. Significant inhibition of biofilm formation was observed at concentrations of **0.156% (v/v), 0.078% (v/v), and 0.039% (v/v)**.

However, the essential oil **did not exhibit significant antibiofilm activity against *Streptococcus mutans* and *Enterococcus faecalis*** under the experimental conditions tested. This suggests that the antibiofilm efficacy of Sea buckthorn oil may vary depending on bacterial species and their biofilm-forming mechanisms.

The results obtained from microtiter plate assays and ELISA plate readings confirmed the **selective antibiofilm activity of Sea buckthorn oil**, particularly against *E. coli*. The reduction in optical density values indicated a decrease in biofilm biomass compared with untreated control samples.

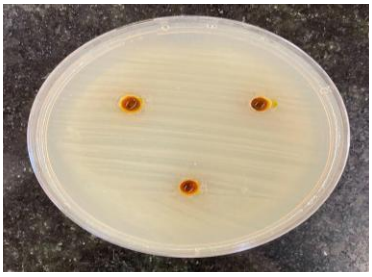

Image 1: Growth on Muller Hinton Agar

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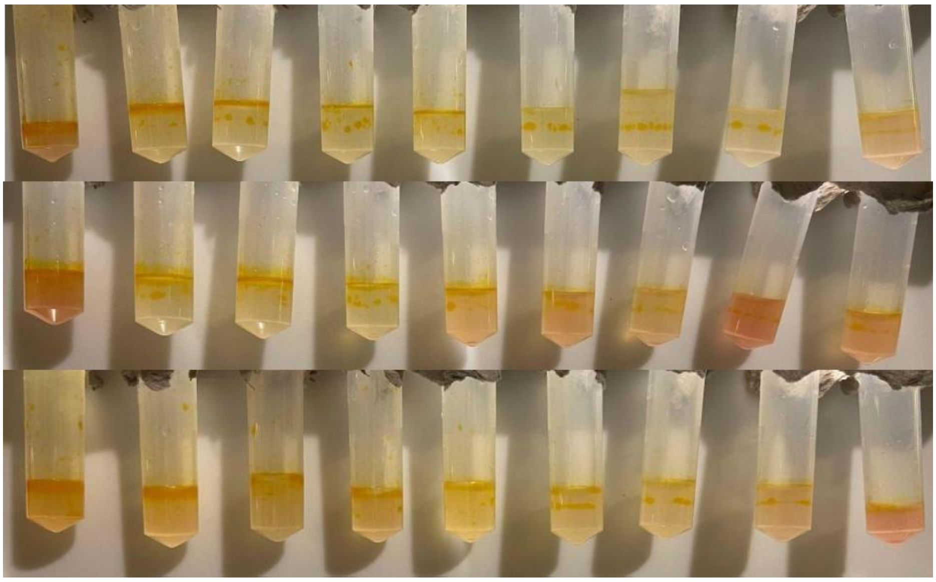


Image 2, Image 3, Image 4: Cuvettes showing MIC of Sea buckthorn essential oil for E.coli., E. faecalis., and St. mutans

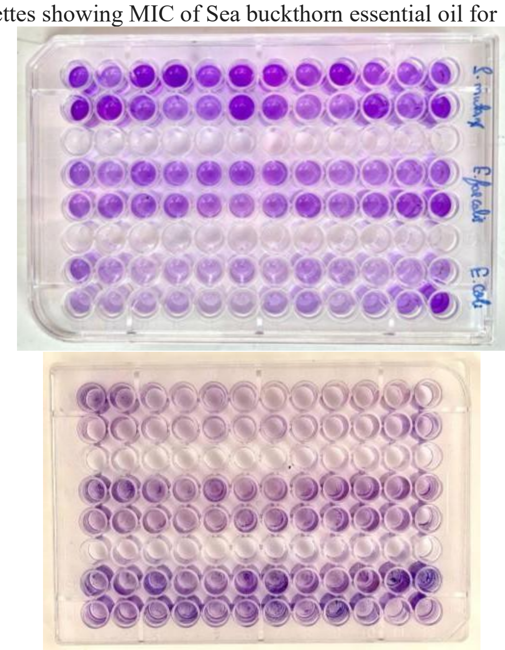
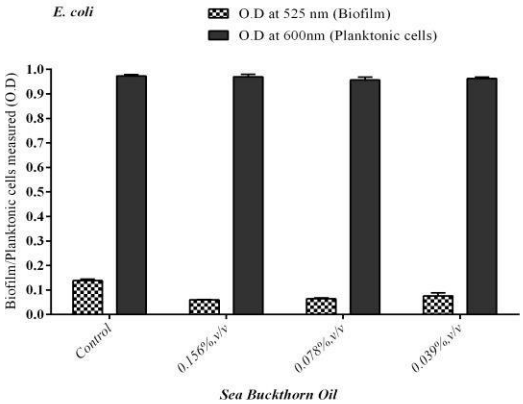


Image 4, Image 5: Microtitre plates showing anti-biofilm activity of Sea buckthorn essential oil against E.coli., E. faecalis., and St. mutans



Graph 1: Inhibition of Biofilm formation by Sea Buckthorn essential oil.

The zones of clearance were seen for all three species of bacteria only at the highest concentration of 40 µl. At the lowest concentration level of 0.312%, v/v, Sea buckthorn potentially inhibited the bacterial growth of E. coli. At the lowest concentration level of 0.625%, v/v, Sea buckthorn potentially inhibited the bacterial growth of E. faecalis. At the lowest concentration level of 1.5%, v/v, Sea buckthorn potentially inhibited the bacterial growth of St. mutans. Sea Buckthorn oil potentially inhibited biofilm formation for E. coli at lowest concentration levels of 0.156%, v/v, 0.078%, v/v and 0.039%, v/v, respectively. It did not show any antibiofilm activity against St. mutans and E. faecalis.

Discussion

Sea buckthorn fruits have been used in ancient Indian, Chinese, and Tibetan medicine for dysfunctions of the alimentary, respiratory, and circulatory system. Sea buckthorn oil used internally has positive effects on the digestive system lowering inflammation (19). Oral application is adjuvant in the treatment of gastric, duodenal, and intestinal ulcers can also be treated effectively. It has been shown to reduce inflammation processes in the vagina and cervix. A high amount of vitamin C makes it suitable for immune deficiencies; due to its antioxidant activity, it removes free radicals and strengthens the immune system (20).Previous studies by Beata Olas, 2018 (21) have demonstrated the cardio protective effects of Sea buckthorn. Studies by Żuchowski et al., 2022 (22) have reported the usage of Sea buckthorn for its cosmetic and anti-aging properties. Sandra Urbaniak et al., 2014 (23) stated that Sea buckthorn can act as a treasury or reservoir for Vitamin C (Ascorbic Acid), thus be used as a replacement therapy for acute scurvy. Studies by Zhang J, 2021 (24), analyzed the immunoenhancement effects of Sea buckthorn oil in mice.

Conclusion

Sea Buckthorn essential oil potentially inhibited the microbial growth and biofilm formation by E. coli, St. mutans and E. Faecalis. Overall, the experimental findings demonstrate that **Sea buckthorn essential oil possesses significant antimicrobial activity against pathogenic bacteria and selective antibiofilm properties**. The oil effectively inhibited bacterial growth and biofilm formation in *E. coli*, suggesting its potential application as a natural antimicrobial agent in pharmaceutical, dental, and biomedical formulations.

These findings support the potential use of **Sea buckthorn oil as a natural therapeutic agent for preventing microbial infections and controlling biofilm-related diseases**, particularly in oral health applications.

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