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ANTI-BIOFILM AND ANTIMICROBIAL ACTIVITIES OF SELECTED MEDICINAL PLANTS ETHANOL AND AQUEOUS EXTRACTS AGAINST *STREPTOCOCCUS MUTANS, PORPHYROMONAS GINGIVALIS* AND *CANDIDA ALBICANS*

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Abstract *The current investigation was carried out to determine the total polyphenol and total flavonoids of Camellia sinensis, lemongrass, rosemary, and Sea buckthorn aqueous and ethanol extracts and its antibiofilm (Crystal Violet Staining Assay), antimicrobial activity (well diffusion and micro-dilution techniques) and viable cell inhibition assay against oral infection causing microbes by good diffusion and micro-dilution techniques. The results showed that the total polyphenol compounds in the ethanol range were (55±00-150±0.5 mg GAE/g sample) and the aqueous extracts range was (50±0.4-110±0.2 mg QE/g of sample). The total flavonoids in the ethanol were found in the range (24±0.1-24±0.1 mg GAE/g sample) and the aqueous extracts were found in the range (20±0.1-70±0.2 mg QE/g of sample). The maximum zone of inhibition was 20±0.1mm, and 19±0.6mm was observed against Streptococcus mutans by ethanol extracts of Camellia sinensis and lemongrass respectively. The lowest zones of inhibition 09±0.1mm and 10±0.4mm were shown in Sea buckthorn aqueous extracts against Candida albicans and Porphyromonas gingivalis. The range of MIC (mg/mL) for Camellia sinensis (3.9-62.5), lemongrass (3.9-125), rosemary (15.6-250), and Sea buckthorn (500-1000) against the tested microbes. The two (02) biofilm development stages were investigated, which were the prevention of biofilm attachment (T0) and destruction of 24h pre-formed biofilm (T24). It was found that maximum biofilm inhibition (T_0) was found 85±0.1% and that maximum biofilm disruption (T_{24}) was found in aqueous extract treatment (65±0.3%) against Streptococcus mutans. Therefore, the studies of plant extracts have the latent utilization in the synthesis of mouthwash, toothpaste, and other drugs associated with numerous mouth diseases.*

Keywords*:* *Seabuckthorn leaves; lemongrass; rosemary; oral pathogens; antibacterial activity; anticandidal activity; bioactive compounds*

Introduction

Biofilms are intricate bacterial communities composed of multiple species that form in the oral cavity as plaque. These biofilms are associated with the development of dental cavities and gum diseases (Muhammad et al., 2017). The method of synthesis of biofilms is irreversible and energetic. It engages microbial adhesion to the surface, micro- and macromolecule adsorption, preparation of EPS, discharge, synthesis of colony, full-grown, and dispersal of biofilm. While a similar procedure takes place frequently, it is also known as cyclic. The adult biofilms are one thousand (1000) times extra opposed to planktonic microbes and safeguard their cultures from the shear force, mechanical stress, pH change, and nutritional shortage (Gebreyohannes et al., 2019; Rodis et al., 2021; Sharma et al., 2019).

Therapeutic herbs and botanicals have been utilized to cure many ailments since the very old era. Medicinal plant extracts employed in conventional medicine recognized a bulky undiscovered source. In the past, innovative therapeutic substances have been produced from plants. They produce significant molecules with latent utilization in therapy. Their elevated bio-efficacy makes them effective in curing various infections produced by microbes. Current findings have targeted antibiofilm and antimicrobial potential. Botanical substances could harm the microbes by intermingling with their cell membrane ingredients, quorum sensing, and proteins. Additionally, exploration of the antibiofilm characteristics of these molecules has observed that in addition to their bactericidal and fungicidal properties, disruption of biofilm could also be an outcome of the medicinal plant extracts (Ali et al., 2025; Guimarães et al., 2021; Lahiri et al., 2019).

Periodontal diseases and dental caries are important health issues of humans and happen irrespective of socioeconomic group; while mostly occur in deprived populations (Rodrigues et al., 2000; Petersen and Ogawa, 2012). Periodontal diseases are common inflammatory ailments with the oral cavity synthesized by a complex biofilm of inhabitant periodontopathic and commensal microbial species

which create a vital branch of diseases along with the host-linked environment and factors (Jayanta et al., 2014). Teeth and gum illness have been linked with systemic chronic diseases like heart disease (Meurman and Hamalainen, 2006). The development of dental caries is created through the accumulation and colonization of oral microbes, which stick and colonize the teeth' face, and lastly with other germs that create teeth plaque ailments (Jayanta et al., 2014). Although enormous progress has been made in the world of dental health position, to date one of the most prevalent diseases is dental caries (Van, 2008). A cariogenic bacterium *Streptococcus mutans* produces an acid, which is a byproduct of sugar metabolism causing the initial stage of dental caries to be differentiated by damage to surface teeth arrangements. A Cariogenic bacterium causes the colonization of teeth which is one of the more significant danger aspects in the formation of dental illness (Loesche, 1986). *Candida albicans* and *Streptococcus mutans* are the common microbes mostly connected with oral cavity illness; *C. albicans* is the main ordinary cause of lungs, nails, skin, septicemia, endocarditis, vaginitis, and oral thrush infections (Preeti and Udav 2014). In many countries dental treatments are costly and not simply reachable; as a result, communities have bowed to the utilization of herbs in the form of miswak or composition of toothpaste to care for the teeth from infections (Shan et al., 2007; Lee et al., 2011). In many developing countries diverse herbal species of therapeutic significance have effectively been utilized including in toothpastes and mouthwashes (Jayanta et al., 2014). There is a need to screen medicinal plants for their promising biological activity. Furthermore, dental care companies and community research plans are studying therapeutic herbs and their tinctures for their capability to manage the microbes, which are the main source of mouth illness. The current study is designed to evaluate the phytochemical screening and antimicrobial activities of Seabuckthorn leaves against oral cavity microbes.

Materials and methods

Collection of Plant Materials and Extraction

The healthy developed *Hipphophae rhamnoides* Linn (Sea buckthorn) leaves, *Camellia sinensis* (Black tea), *Cymbopogon Citratus* (Lemon Grass) and *Rosmarinus officinalis* L. (Rosemary) leaves were collected from Food Technology Center of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar-Pakistan. To remove any dust the leaves were faintly cleaned with drinking water and kept in the shade to dry. The dehydrated leaves were ground using a laboratory mill to create a fine powder, which was then stored in a sealed, sterilized brown bottle for future experimental use. The extraction process for the leaves was conducted following the procedure outlined (Javid and Bashir, 2015).

Quantification of Bioactive Components

Determination of Total polyphenol and Total flavonoids assay were determined using the reported methods (Priyanka et al., 2012).

Antimicrobial Assay

Tested oral microorganism

The *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans* were collected from the Microbiology Research Section of PCSIR Laboratories Complex Peshawar, Khyber Pakhtunkhwa, Pakistan. The *Streptococcus mutans* strain was plated onto brain heart infusion agar enriched with 1% yeast extract (BD) (BHIY) and incubated at 37°C with 5% Carbon dioxide for 48 hours in a CO_2 incubator. Afterward, a single colony was transferred to BHIY broth and cultured at 37°C with 5% CO_2 for 16 hours. For *Porphyromonas gingivalis*, the strain was plated onto ABHK agar and cultivated under anaerobic circumstances at 37°C for two to ten days. A single colony was then inoculated into BHIY broth supplemented with hemin (5 mg/mL) and menadione (0.5 mg/mL), followed by incubation under anaerobic conditions at 37°C forty-eight hours. Potato Dextrose Agar (PDA) was used for *Candida* albicans cultures. These cultures were stored in a cooled incubator (Memmert, Germany) for seven days to allow for re-culturing.

Antimicrobial activities

The zone of inhibition (ZI) and Minimum Inhibitory Concentration (MIC) of herbal extracts were found according to the mentioned methods (Bashir and Javid, 2013).

Anti-biofilm Assay

Inhibition of Bacterial Biofilm Formation

Two biofilm growth ways were evaluated, which were inhibition of biofilm attachment (T0) and damage of pre-formed (24 hrs) biofilm. The biofilm was permitted to be made for either 0 hrs (T0) or 24 hrs (T24) before adding plant extracts at an ending dosage of 500mg/mL. For the T0 experiment, 100µL of each bacterial culture (OD_{590} = 0.02 equivalent to 1.0×10^6 CFU/mL) synthesized in Tryptone Soy Broth (TSB) was shifted into sterilized flat-bottomed 96-well microtitre plates go after by addition of 100µL of the plant extract and kept in an incubator (Memmert-Germany) at 37°C for 24hrs. For T24, 100µL of standardized microbial cultures were pre-incubated for 24 hrs for the growth of biofilm, before plant extracts addition. For T24 and T0 suitable controls were prepared: Negative control (TSB + culture), Positive control (TSB + culture + antibiotics). Following incubation (24 Hrs), the modified crystal violet staining (CVS) procedure was applied to quantify the biomass of biofilm (Olawuwo et al., 2022).

Crystal Violet Staining (CVS) Assay

As described above next to incubation, the wells were gently poured out and the plates were washed at least 03 times with autoclaved distilled H_2O to eliminate loosely or unattached cells. The plates were air dried and afterward for 45 minutes at 60°C kept in an oven (Memmert-Germany). After that 96% methanol (150 µL) was shifted to the wells for 15-20 min to stick adherent cells. The plates were drained and the remained cells were stained at room temperature for 20 min with 100 µL of 0.1% crystal violet solution. The surplus strain was cleaned off by washing the plates at least 05 times with H_2O. As a result, the biomass of biofilm was assessed semi-quantitatively by re-dissolving the crystal violet stain ties to the sticky cells with 150µL of ethanol (100%) to the wells destain. The plate's absorbance was observed at 590nm by applying an Absorbance Microplate Reader (BioTek- Romania) following gentle and careful shaking. The average Optical density (OD_{590}nm) of the samples was noted and the results were expressed as inhibition percentages by applying the equation below (Olawuwo et al., 2022).

Percentage (%) inhibition = ODNegative control - ODSample x 100

ODNegative control

$$\text{Percentage (\%) inhibition} = \frac{\text{ODNegative control - ODSample}}{\text{ODNegative control}} \times 100$$

Statistical analysis

The resulting data were expressed as an average ± standard deviation (SD) and calculated by applying software (SPSS). Significance statistical was calculated with a p-value of < 0.05.

Results

The bioactive compounds in selected plant extracts are shown in Fig. 1. The results showed that ethanol extracts of *Camellia sinensis* observed the maximum total polyphenol i.e. 150±0.5 mg GAE/g sample, followed by 95±0.2 mg GAE/g sample, 60±0.1 mg GAE/g sample and 55.00 mg GAE/g sample in Lemongrass, Rosemary and Sea buckthorn respectively. The total flavonoids data showed that the maximum was 80±00 mg QE/g of sample and the minimum 24±0.1 mg QE/g of sample was calculated in Sea buckthorn. The aqueous extracts of *Camellia sinensis* showed maximum total polyphenols 110±0.2 mg GAE/g sample, followed by 90±0.5 mg GAE/g, 60±0.1 mg GAE/g and 50±0.4 mg GAE/g in lemon grass, rosemary and sea buckthorn respectively. The maximum total flavonoid was 70±0.2 mg QE/g of sample in *Camellia sinensis* and the minimum was 20±0.1 mg QE/g of sample in Sea buckthorn.

Antibacterial activities (Zone of Inhibition in millimeters) of plant extracts are shown in Fig 2. The antibacterial activities of *Camellia sinensis* showed a maximum zone of inhibition 20±0.1mm against *Streptococcus mutans*, 18±0.5mm against *Porphyromonas gingivalis*, and 16±0.2mm against Candida albicans by ethanol extract and this plant showed a lower zone of inhibition 17±0.4mm, 14±0.1mm and 12±0.5mm against *Streptococcus mutans*, *Porphyromonas gingivalis* and Candida albicans respectively by aqueous extracts. The lemongrass ethanol extracts observed a maximum zone of inhibition of 19±0.6mm against *Streptococcus mutans*, followed by 17±0.1mm and 15±0.2mm against *Porphyromonas gingivalis* and Candida albicans respectively. This plant's aqueous extracts findings revealed that it has less zone of inhibition i.e. 16±0.1mm (*Streptococcus mutans*), 13±0.2mm (*Porphyromonas gingivalis*), and 11±0.4mm (*Candida albicans*) as compared to ethanol extracts. The rosemary ethanol extracts demonstrated a minimum ZI of 14±0.1mm against *Candida albicans* and a maximum ZI of 18±0.4mm against *Streptococcus mutans*. The water extracts ZI found in the range of 10±00mm- 15±0.2mm of the study microbes. The Sea buckthorn extracts illustrated low ZI as compared to the mentioned plant extracts. This can be explained that they have found low bioactive compounds as compared to the rest of the plants. The ZI demonstrated that ethanol extract is the more efficient solvent extract as compared with water extract. The MIC (mg/mL) of plant extracts are shown in Fig. 3. The MIC range of the *Camellia sinensis* was (3.9-62.5 mg/mL), Lemongrass was (3.9-125mg/mL), Rosemary was (15.6-250mg/mL) and Sea buckthorn was (500-1000 mg/mL). The results indicated that the *study plant* extract showed MIC activities at variable degrees against oral microbes.

Anti-biofilm activity of the study plant extracts against selected pathogens is shown in Table 1. Results for the antibiofilm activities are classified into two (02) stages consequent to the developmental phases of biofilm. Initially, the early connection of biofilms is represented at T_o and biofilm formation (T_{24}) at 24 h. The results are presented in Table 1 and are interpreted either as weak antibiofilm activity (0–49%) or good antibiofilm activity (50–100%). The maximum antibiofilm I% (T_0) showed by ethanol extracts of *Camellia sinensis* against *Streptococcus mutans* was 85±0.1%, followed by 83±1.5% and 80±01% against *Porphyromonas gingivalis* and *Candida albicans* respectively. This extract's anti-biofilm inhibition activity (T_{24}) was 65±0.3%, 63±01%, and 60±01% against Streptococcus *mutans*, *Porphyromonas gingivalis*, and *Candida albicans* respectively. If we see the aqueous extracts of Camellia sinensis have found a low antibiofilm I% inhibition (T_o and T_{24}) as compared to the ethanol extracts. The lemongrass ethanol extracts showed 77±0.4%, 73±0.1%, and 70±01% antibiofilm activities (T_0) against Streptococcus *mutans*, *Porphyromonas gingivalis*, and *Candida albicans* respectively. Similarly, T24 antibiofilm activities of aqueous extracts have been found 60±1.3%, 57±0.6%,

and 54±00% against Streptococcus *mutans*, *Porphyromonas gingivalis*, and *Candida albicans* respectively. It was found that the aqueous extract antibiofilm (T_o) observed 68±0.2%, 64±0.3% and 62±1.3% against Streptococcus *mutans*, *Porphyromonas gingivalis* and *Candida albicans* respectively and T_{24} antibiofilm activities were lower as compared to T_0. At the initial cell attachment stage (T_0), the ethanol extracts of rosemary had antibiofilm inhibitory activity with 72±1.5 %, 70±01%, and 65±0.3% reduction in cell attachment against *Streptococcus mutans*, *Porphyromonas gingivalis* and *Candida albicans* respectively. The cell attachment after 24 hours (T24) was found to be 56±0.5, 52±1.2, and 50±0.5 against *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans* respectively. The rosemary aqueous extracts antibiofilm T_0 were 62±0.3%, 60±1.1%, and 57±0.2% against *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans* respectively. The maximum antibiofilm T_{24} of rosemary aqueous extract maximum was low values as 51±0.1%, 47±00%, and 45±00% against *Streptococcus mutans*, *Porphyromonas gingivalis* and *Candida albicans* respectively. The Sea buckthorn ethanol extracts initial antibiofilm (T_0) maximum value was 55±0.4% (*Streptococcus mutans*) and minimum value was 50±1.1% (*Porphyromonas gingivalis*), while moderate value was 53±1.2% (*Candida albicans*) and the antibiofilm (T_{24}) displayed as lower values as (50±00% *Streptococcus mutans*, 48±01% *Porphyromonas gingivalis* and 45±0.1% *Candida albicans*) compared with their corresponding T_0. The Sea buckthorn aqueous extracts confirmed maximum T0 was 52±0.5% (*Streptococcus mutans*) and the lowest was 48±0.5% (*Candida albicans*). The Sea buckthorn aqueous extracts T_0 and T_{24} proved maximum values were (52±0.5% and 45±0.2%) and minimum were (48±0.5% and 40±0.1%) against *Streptococcus mutans* and *Candida albicans* respectively.

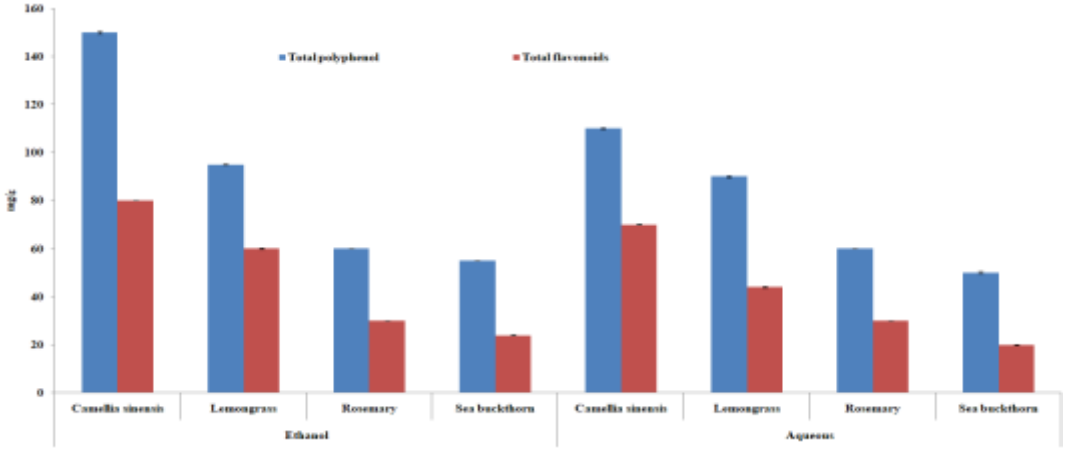

Figure 1. Bioactive Components in Selected Plants Extracts

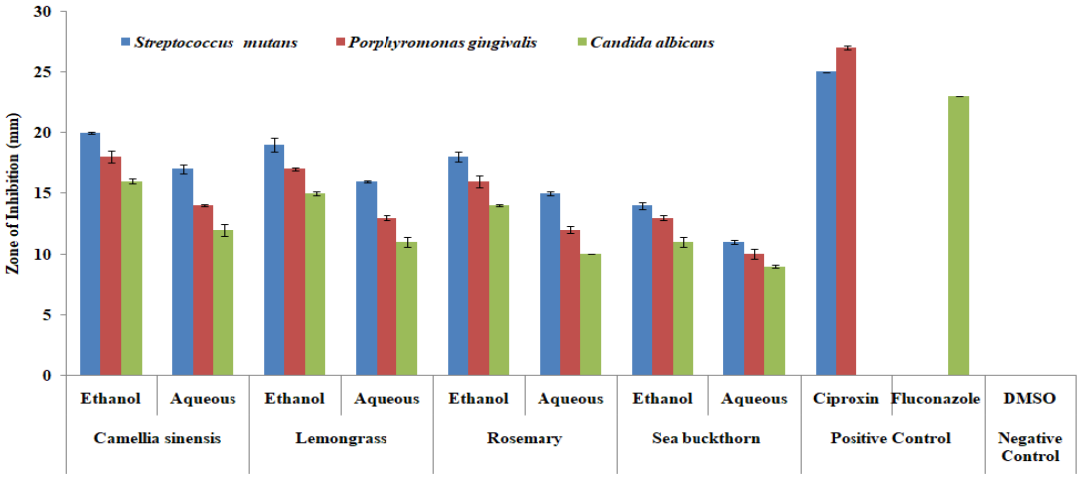

Figure 2. Antibacterial Activity Zone of Inhibition (mm) of Plants Extracts

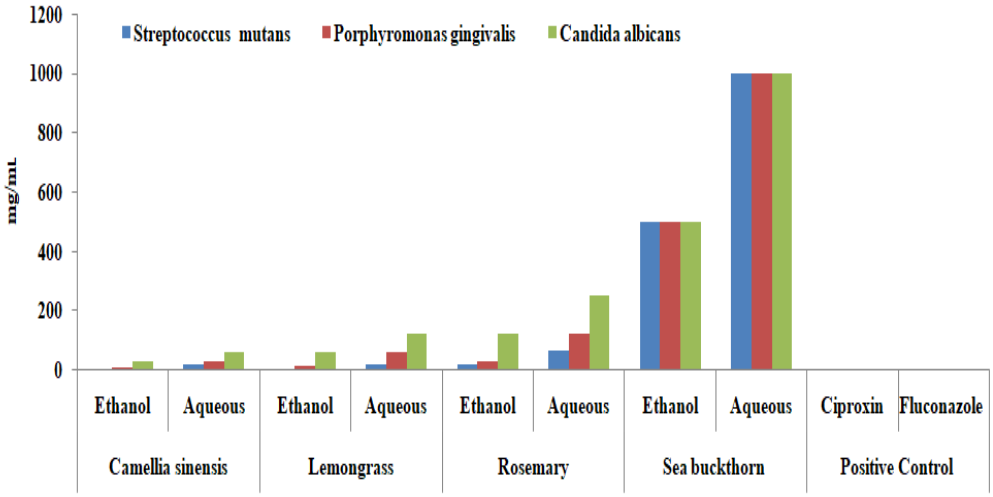

Figure 3. MIC (mg/mL) of Plants Extracts

Table 1. Anti-biofilm activity of plant extracts against selected pathogens

Plants	Extracts		% inhibition		
			Streptococcus mutans	*Porphyromonas gingivalis*	*Candida albicans*
Camellia sinensis	Ethanol	T_0	85±0.1	83±1.5	80±01
		T_{24}	65±0.3	63±01	60±01
	Aqueous	T_0	74±0.7	70±0.2	68±0.1
		T_{24}	57±0.5	52±0.5	50±1.2
Lemongrass	Ethanol	T_0	77±0.4	73±0.1	70±01
		T_{24}	60±1.3	57±0.6	54±00
	Aqueous	T_0	68±0.2	64±0.3	62±1.3
		T_{24}	54±00	51±0.1	50±0.1
Rosemary	Ethanol	T_0	72±1.5	70±01	65±0.3
		T_{24}	56±0.5	52±1.2	50±0.5
	Aqueous	T_0	62±0.3	60±1.1	57±0.2
		T_{24}	51±0.1	47±00	45±00
Sea buckthorn	Ethanol	T_0	55±0.4	53±1.2	50±1.1
		T_{24}	50±00	48±01	45±0.1
	Aqueous	T_0	52±0.5	50±0.1	48±0.5
		T_{24}	45±0.2	43±00	40±0.1
Positive Control amoxicillin		T_0	99±00	99±00	NA
		T_{24}	99±00	99±00	NA
Fluconazole		T_0	NA	NA	92±00
		T_{24}	NA	NA	95±00
Negative Control (culture + TSB)		T_0	00	00	00
		T_{24}	00	00	00

NA=Not applied.

Discussion

Bacterial Pathogens Responsible for Dental Caries and Periodontal Diseases

Without a prescription, medical tests, and the use of commercial antibiotics extensively, the pathogenic microbes of humans have developed resistance to these antibiotics. This situation has prominent terror in various developed and underdeveloped countries and scientists are forced to discover an alternative solution to these drugs, such as isolated from herbal and natural sources. It is a reality that *In-Vitro* evaluation of plant species with conventional medication capability is the basic step towards the synthesis of eco-friendly and effective drugs against ailments. In the present study, a similar move was assumed to investigate potent healing compounds from *Camellia sinensis*, Lemon grass, Rosemary, and Sea buckthorn aqueous and ethanol extracts for the

management of different dental caries and periodontal inflammatory diseases that are caused by pathogenic microbes. The massive utilization of usual antimicrobials for curing oral diseases has fueled the appearance of antimicrobial confrontation. This opposition is rising more rapidly than the synthesis of novel medicines, showing significant issues in healthcare (Alam et al., 2023). Dental ailments are global health problems having noteworthy outcomes on patient quality of life and function. It is observed that the incidence of dental caries and periodontal disease will rise constantly due to high sugar intake in food, inadequate consumption of fluorides in food, and less care of teeth (Petersen et al., 2005). The *Streptococcus mutans* is a chief contributory agent of dental carries, which is a part of endogenous oral microorganisms. Streptococcus mutans produce the enzymes, which discharge a little chain of carboxylic acids (fermentation byproducts) resulting in teeth dematerialization and the formation of teeth cavities (Sriram et al., 2014). The antimicrobial activities of flavonoids are chiefly probable because of their capabilities to synthesize a composite with bacterial cell walls as well as compounds with extracellular and soluble proteins (Preeti et al., 2014). The noteworthy antimicrobial activities of the plant extracts against oral and teeth ailment microbes might have been accredited to the fact that bioactive compounds responsible for the antibiotic potency are commonly arrested in the solvents and these phytochemicals compounds may be able to go into the plump cell wall in the way of usual diffusion rout synthesized by microbial porins (Rosas et al., 2012; Shan et al., 2007; Gao et al., 1999; Benz and Bauer 1988), and alter the microorganisms enzymes like gingipain that are responsible for survival and virulence of *Porphyromonas gingivalis* resulting lysis of cells (Jayanta et al., 2014).

The MIC of green tea extract was found 0.2% against S. mutans and against L. acidophilus was 0.3%. The parallel MBC values were against S. mutans was 0.8% and against L. acidophilus was 0.9%. In the 30 µL ethanol green tea extract (300 µg) the zone of inhibition against S. mutans was 18.33mm and against L. acidophilus the zone of inhibition was 12.67mm (Anita et al., 2014).

Anti-Biofilm Activities

Medicinal herbs are a vital foundation of organic substances having ant-biofilm characteristics, accredited to their wealthy occurrence of bioactive compounds (Mastoor et al., 2022). Biological active compounds have reported anti-biofilm activities through numerous phenomena, such as reducing virulence factors, disrupting extracellular matrix synthesis, preventing cell attachment, inhibiting cell attachment, and inhibiting the synthesis of polymer matrices. Natural substances in herbal extracts have been observed to change surface properties like as hydrophobicity, hydrophilicity, roughness, and texture, which assist in inhibiting biofilm formation and bacterial adhesion (Rachidatou et al., 2024). The plant extracts anti-biofilm activities could be accredited to the distinctive capabilities of these metabolites.

A common dental disease (Dental caries), is strongly connected to the microbes that build up on the teeth surface. Mutant's streptococci are predominantly significant in the growth of dental biofilm and the beginning of dental carries, the causative agents are; *Streptococcus ferus*, *Streptococcus rattuce*, *Streptococcus cricetus*, *Streptococcus sobrinus* and *Streptococcus mutans*. As an outcome, numerous herbal tea extracts, particularly those extracted from leafy herbs, are thought to not only maintain food safety but also inhibit oral microbial growth (Jungmin et al., 2013). It was reported that the phytochemicals quercetin inhibited 96% *Streptococcus mutans* and Kaempferol inhibited 97% Streptococcus mutans at 8 µg/ml concentrations (Jayanta et al., 2014). It was observed that medicinal plants like *Acacia arabica*, *Tamarix aphylla*, and *Melia azadirachta* displayed antibiofilm against oral microbes (Muhammad et al., 2017). These antibiotic properties were accredited to trouble-free diffusion of therapeutic substances in the microbial cell resulting in cell wall and cytoplasmic membrane disruption (Jayanta et al., 2014).

Bioactive Components in Selected Plants

Medicinal plants and herbs are wealthy sources of different compounds such as terpenoids, vitamins, tannins, flavonoids, and phenolic acids, which are accounted for their biological properties. The antioxidant and antimicrobial herbal tea leaves are accredited to their phenolic substances (Jungmin et al., 2013).

Black tea

It was reported that the leaves of C. sinensis (L.) have a wealthy source of volatile compounds, minerals, enzymes,3% amino acids, and phytochemicals possessing 30-40% polyphenols like theaflavins, gallic acid esters, epicatechin, gallocatechin, catechins, and additional polyphenols (Samanta et al., 200). It has been observed that black tea has possessed flavonoids, phenols, and tannins at concentrations of 7.5mg/g, 96mg/g, and 243.75 mg/g respectively (Anita et al., 2014). It was observed that black tea has found flavonoids, phenols, and tannins in a concentration of 350 mg/g, 0.0105 mg/g, and 0.308 mg/g respectively (Subramaniam et al., 2012). Though, the ingredients of these substances may differ considerably based on dynamics like processing techniques, geographical location, climate conditions, cultivation practices, and soil quality (Zhang et al., 2018). Various studies have reported that the tannins

and polyphenols isolated from *Camellia sinensis* could stop the growth of various microbes (Jungmin et al., 2013).

The antibacterial activities of the herbs-derived polyphenols are considered to carry out the damage to the microbial cell membrane, whether functionally or structurally (Jungmin et al., 2013). Scientists have proposed that the conjugated double bonds and hydroxyl groups in leaves of herbal tea extracts could play a role in attaching to ingredients of the microbial cell wall. Plant-origin substances harmfully impact bacterial cells utilizing numerous phenomena, such as hitting the phospholipid bilayer of the cell membrane and enzyme system disturbing (Jungmin et al., 2013).

Rosemary

The Rosemary extracts (100mg/mL0 showed a 12 mm zone of inhibition against *E. coli* (Ahmad et al., 2023). These findings showed dissimilarities in a study carried out in Iran which found that leaf extracts at a concentration (500mg/mL) found a 10.5 mm zone of inhibition (Jiffri and Zahira, 2001). The ethanolic extracts of Rosemary were evaluated by applying 12.5, 25, 50, and 100mg/mL concentrations against *E. coli*. The MIC ranges were 12.5-25mg/mL for isolated strains (Ahmed et al., 2023). These observations were contrasted with the results found in Brazil as calculated MIC was 320mg/mL against E. coli (Genena et al., 2008). Correspondingly, the observation also showed differences from those of a study carried out in Iran, which identified Rosemary extracts 200mg/mL MIC (Golshani and Shaifzadeh, 2014). It was also reported that the Rosemary extracts showed an inhibitory effect against *Candida albicans* at 100 µg/mL concentration (Saeidi et al., 2019). The findings also showed significant antifungal activities against *Candida albicans* of Rosemary extract. These activities could be accounted for by the occurrence of bioactive substances in the extracts, such as flavonoids, phenols, camphor, and 1,8-cineole, all of which hold antifungal activities (Meccatti et al., 2021). The inhibitory effect of Rosemary bioactive substances is accounted for the mutual actions of different little ingredients possessed in both non-volatile and volatile compounds, to a certain extent than the effectiveness of any single ingredient (Tornuk et al., 2011). The Rosemary extract ingredients exertion synergistically, acting together with the microbial cell membrane and controlling reactions like fatty acid transport, cellular leakage, electron transport, nutrient absorption, genetic material synthesis, and fatty acid production. Furthermore, these compounds intermingle with proteins in the membrane, resulting in membrane function and structure disruption (Fung et al., 1977). These dissimilarities might be accounted for by the differences in the cell membrane porosity or extra genetic issues. It was also noted that variations in these findings might be accredited to the dissimilarities in the microbial cell wall shape, structure, Rosemary origin, or the particular plant part investigated in the experiments.

Lemongrass

The leaves of lemongrass leaves are reported to hold various bioactive substances such as flavonoids, esters, ketones, aldehydes, terpenes, alcohols, and phenolic molecules like apigenin, kaempferol, luteolin, quercetin, iso-orientin and tannins (Muala et al., 2021). The *Cymbopogon citrates* extract total phenolic compounds in ethanol extracts (70%) were found 49.317 mg GAE/g and in ethanol extracts (30%) were found 50.017 mg GAE/g. However, both these figures were considerably higher than the total phenol compounds of ethanol extracts (96%) i.e. 43.433 mg GAE/g (Hasim et al., 2015). These findings were lesser than those reported by Sah et al. (2012), who described that the lemongrass leaves ethanol extracts (40%) found 67.28 mg GAE/g total phenols compounds. In dissimilarity, the lemongrass leaves found in methanol and hexane extract 66.94 mg GAE/g and 72.55 mg GAE/g phenolic compounds respectively (Suryanto et al., 2019). It's imperative to remember that total phenol analysis does not distinguish between diverse kinds of phenols (trimer, dimer, monomer) possessed in the extracts (Hasim et al., 2015).

Sea buckthorn

The Sea buckthorn demonstrated antibacterial activities against microbe (Gram-negative), consistent with the findings of Michel *et al*. (2012). Similarly, the finding of Yogendra *et al*. (2013) highlighted the significant antibacterial effects of *H. rhamnoides* leaves extract (SLE) and a phenolic-rich portion (PRF) against such as *E. coli*, *S. typhi*, *S. dysenteriae*, *S. pneumoniae*, and *S. aureus*. The SLE showed the highest ZI (15.23 ± 0.84 mm) against *S. dysenteriae*, while the lowest (8.33 ± 1.12 mm) was observed for *S. pneumoniae*. The PRF exhibited the largest inhibition zone (20.67 ± 1.54 mm) against *S. dysenteriae* and the smallest (8.0 ± 0.47 mm) for *S. typhi*. The study of Maheshwari *et al*., 2011 calculated 319.33 mg GAE/g sample of total phenolic and other phytochemical (isorhamnetin, kaempferol, quercetin, myricetin, and gallic acid) in the range of 1.935–196.89 mg/g of Seabuckthorn leaves. Berries and leaf extracts of Sea buckthorn were shown to have antibacterial properties against methicillin-resistant *Staphylococcus aureus* (Qadir et al., 2016). Additionally, both the hydroalcoholic and aqueous sea buckthorn extracts of leaves demonstrated antibacterial effects against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus* (Upadhyay et al., 2010).

The antibiotic properties of *H. rhamnoides* extracts are likely attributed to their aptitude to synthesize complexes with soluble and extracellular proteins, as well as microbial cell walls, through unfocused interactions covalent bonding, hydrophobic effects, and hydrogen bonding. This suggests that their antimicrobial mechanism may involve inactivating enzymes, adhesions of microbes, and cell membrane-carrying proteins (Eva et al., 2020; Zahid et al., 2025). The antimicrobial mechanisms of polyphenols are primarily related to their ability to destabilize cytoplasmic membranes, alter membrane porosity, extracellular microbial enzymes shutdown, microbial metabolism disturbance, and deprive microbes of essential growth substrates, including key minerals (Sandulachi et al., 2022; Kayani et al., 2025). The phenolic content in plants can be influenced by several factors, including sample preparation (such as drying time and temperature), the plant's growing conditions, the extraction method used, and the analysis technique employed.

Conclusion

The study concluded that black tea, rosemary, lemon grass, and Sea buckthorn extract have the potential to produce natural compounds. The raw extracts demonstrated antimicrobial activity against oral microbes, which may lead to the discovery of novel therapeutic antibiotic compounds that could serve as selective drugs for protecting human and animal health. Additionally, these extracts could provide natural tools for studying infectious diseases. Further isolation of bioactive compounds at a pilot plant scale is necessary to develop various antimicrobial mouthwash products.

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Declaration

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Authors contribution

JA and AH conducted research and wrote the initial draft of manuscript. JA, AH, and JA collected the literature and wrote the manuscript, and edited the manuscript in original. All authors have read and approved the final manuscript. The author have read and approved the final manuscript.

Conflict of Interest

The authors state that there is no conflict of interests with regard to this study. There is no conflict of interest in any financial or personal manner concerning the development of this project, the gathering of data, the interpretation of the data, or the writing and publishing of this paper.

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All authenticated data have been included in the manuscript.

Ethics approval and consent to participate

These aspects are not applicable in this paper.

Consent for publication

Not applicable