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Antibacterial Potential of Pressurised Green Extracts from Dry Heat Processed Herbs against Selected Oral Pathogens

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Abstract

Recent developments in herbal extraction have shifted towards green synthesis methods that prioritise safety, environmental sustainability, and enhanced efficiency. This study aimed to compare pressure-treated aqueous extracts from dry-heat-processed *Syzygium aromaticum* (Sa) and *Piper nigrum* (Pn) with their traditional decoctions to develop a high-quality natural antimicrobial formulation for oral health applications. Decoctions (*Syzygium aromaticum* -SaD, *Piper nigrum*-PnD) and pressure-treated aqueous extracts from dry-heat-processed *Syzygium aromaticum*-SaP and *Piper nigrum*-PnP were prepared and evaluated for organoleptic, physicochemical, stability, and phytochemical characteristics. Quantitative assays measured total phenolics, flavonoids, eugenol, and piperine. GC-MS (Gas Chromatography-Mass Spectrometry) identified major bioactive constituents. Antimicrobial efficacy was assessed against *Streptococcus mutans*, *Enterococcus faecalis*, *Porphyromonas gingivalis*, and *Treponema denticola* using inhibition zone, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) tests, biofilm inhibition, protein and cytoplasmic leakage assays. Pressure-treated extracts showed higher yields (SaP:18.03%, PnP:17.56%) and greater phytochemical content, including elevated phenolics (SaP:65.94 mg Gallic Acid Equivalents (GAE)/g; PnP:53.12 mgGAE/g) and flavonoids (SaP:125.23 mg quercetin equivalents (QE)/g; PnP:114.54 mg QE/g) compared to decoctions. Antimicrobial activity was stronger in pressure-treated extracts, with SaP showing larger inhibition zones (16.33 mm for *Streptococcus mutans*), lower MICs (6.67-8.00 mg/mL), MBCs (8.00-10 mg/mL), and higher biofilm inhibition (77.08%). Greater protein and cytoplasmic leakage values for SaP and PnP confirmed enhanced membrane-disruptive effects.

Major Findings: The pressure-treated extracts yielded phytochemically richer, more stable, and more potent antimicrobial herbal preparations compared with traditional decoctions and show strong promise as natural, safe, and effective agents for oral hygiene formulations and oral infection control.

Keywords: Decoctions, Dry-Heat-processed, Oral Health, *Piper nigrum*, Pressure-treated Aqueous Extract, *Syzygium aromaticum*

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1. Introduction

Oral health is now a major priority across the world, and with the WHO supporting safe, affordable traditional remedies, using medicinal plants is emerging as a feasible option for prevention and care[1]. Oral pathogens that cause cavities and gum disease can form biofilms, damage tissues, and spread through the bloodstream, contributing to systemic issues like heart, metabolic, and lung disorders[2,3]. Herbal oral remedies are increasingly used in dentistry for their safe, affordable antioxidant, anti-inflammatory and antimicrobial benefits, though better product standardisation and further research are still needed[4]. Indian kitchen spices, backed by traditional wisdom and scientific evidence, offer safe, natural antimicrobial benefits for oral health, making them strong alternatives to chemically formulated products[5]. The natural vegetal products have the potential to initiate the discovery of new and more effective antimicrobial agents in the future[6,7].

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S. aromaticum (clove; Sa) and *P. nigrum* (black pepper; Pn), which are among the long-used spices in Indian folk medicine, show strong potential as natural agents for managing oral diseases[8]. Recent studies show these spices offer antimicrobial, anti-inflammatory, and antioxidant benefits that help control oral pathogens and support dental health[9]. Wide antimicrobial activity with pronounced anti-oxidant, anti-inflammatory, and analgesic properties due to the presence of bioactive compounds like eugenol, gallic acid, flavonoids and other phenolics present in *S. aromaticum*, render it a good candidate in therapeutic and oral care formulations[10,11]. Clove decoctions contain potent antioxidants and antimicrobials like gallic acid, quercetin, phenolics, and terpenes that reduce inflammation, combat microbes, and protect against oxidative stress[12].

Piper nigrum, the 'King of Spices', native to South India, has been profoundly used as medicine traditionally, for digestive support and the management of various diseases like respiratory infections and gastrointestinal disorders[13]. Its alkaloids, piperine, piperanine, and piperlonguminine provide strong antioxidant, anti-inflammatory, and antimicrobial effects by inhibiting essential protein and nucleic acid synthesis[14]. The chief alkaloid of black pepper, piperine, has prominent antioxidant and anti-inflammatory potential to protect oral tissues with its modulating cellular signaling mechanisms[15,16]. Black pepper aqueous extracts, rich in phenols, flavonoids, and alkaloids, provide strong antioxidant, anti-inflammatory, and antimicrobial effects by neutralising free radicals, regulating inflammatory pathways, and disrupting microbial membranes[17,18]. Besides, the interference of microbial energy metabolism is observed with the interference of important enzymes of critical biochemical pathways by piperine[19,20].

With rising antibiotic resistance, plant-based agents like clove and black pepper have proven to be safe and effective alternatives to chemical mouthwashes for controlling oral pathogens and inflammation[21-24].

Although traditional aqueous extract methods like decoction are safe but less efficient, advanced pressure-treated aqueous extraction from dry heat-processed herbal material now enables higher bioactive yield by keeping water at high temperatures and preventing compound loss[25]. However, the studies on antibacterial efficacy of pressure-treated aqueous extracts from dry heat-processed clove and black pepper are very limited, specifically against oral pathogens. Hence, this study aimed to evaluate the antibacterial potentiality of these extracts against salivary *Streptococcus mutans (S. mutans), Enterococcus faecalis (E. faecalis), Porphyromonas gingivalis (P. gingivalis)* and *Treponema denticola (T. denticola)* in comparison with their conventional decoctions, and provide a practical, safe, and cost-effective approach to natural oral care.

2. Materials and Methods

2.1 Sourcing of Herbal Materials

S. aromaticum buds and *P. nigrum* seeds were obtained from reputable local markets in Tirupati, India and their identities confirmed by a plant taxonomist. The spices were cleaned with distilled water, shade-dried to preserve bioactive compounds, ground to uniform powders in a cleaned and sterilised laboratory grinder, and stored in airtight containers at room temperature, away from direct sunlight, until use.

2.2 Estimation of Ash Content in *S. aromaticum* and *P. nigrum* Powders

Four grams of each herbal powder were incinerated in pre-weighed crucibles at 600°C until white ash formed, then cooled and reweighed to calculate total ash per gram of air-dried material.

2.3 Preparation of Herbal Extracts

The herbal extracts of *S. aromaticum* and *P. nigrum* were prepared by decoction and pressure-treated aqueous extract methods. Decoctions were prepared by boiling 1 g of herbal material in 100 mL of distilled water at 100°C for 20 min, while dry heat and pressure-treated aqueous extracts were obtained by roasting the herbs over a medium flame (150-180°C) for five min in a thick-bottomed pan with continuous stirring until a characteristic roasted aroma was observed. This temperature range aligns with traditional culinary roasting practices for spices and has been documented in prior phytochemical studies[26]. Then autoclave the same ratio of plant material and water as decoction at 121°C temperature and at a pressure of approximately 15 psi (1.05 bar) for 20 min to facilitate pressure-treated aqueous extraction[27]. Both liquid and dry extracts of decoctions and pressure-treated extracts from dry heat-processed clove buds and black pepper seeds were stored at 4°C until use.

2.4 Estimation of Liquid Herbal Extract Concentration and Yield of Dry Extract

Final filtrate volumes of extracts were recorded, and 20 mL from each was dried to obtain the dry extract. The extract concentration (g/mL) was calculated by dividing the dry weight by 20 mL, and the percentage yield was also determined accordingly. The liquid extracts were subsequently diluted to concentrations ranging from 20 to 150 µg/mL for further use.

2.5 Physicochemical Characterisation

Organoleptic traits (colour, odour, taste, appearance, texture) were assessed, while pH, viscosity, soluble mass, and weight per mL were measured to evaluate the usability and quality of the liquid extracts.

2.6 Stability Assessment

The stability of the formulated liquid extracts was evaluated over a period of four weeks under two storage conditions, ambient temperature and refrigeration at 4ºC as per the standard guidelines[28]. The extracts were stored in sealed containers and inspected for changes in colour, odour, overall appearance, pH and viscosity at various time intervals. In addition, the existence of microbial growth was determined by measuring the optical density[29]. Optical density measurements were corrected using extract-matched blanks to minimise interference from inherent colour and particulate matter.

2.7 Phytochemical Analysis

Preliminary phytochemical analysis was done using standard tests, Dragendorff's and Mayer's for alkaloids, Shinoda and alkaline tests for flavonoids, ferric chloride and gelatin tests for tannins, foam test for saponins, and ferric chloride and lead acetate tests for phenolics[30].

2.8 Estimation of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The total phenolic content of the extracts (SaD - *S. aromaticum* decoction, SaP - Pressure-treated aqueous extract from dry heat-processed *S. aromaticum*, PnD - *P. nigrum* decoction, and PnP- Pressure-treated aqueous extract from dry heat-processed *P. nigrum*) was estimated using the Folin Ciocalteu method. A calibration curve was prepared with gallic acid as the reference standard. The extracts were mixed with Folin Ciocalteu reagent and sodium carbonate, and the absorbance was recorded at 765 nm using a spectrophotometer. Results were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of extract[31].

The total flavonoid content of the extracts was estimated using the aluminium chloride colorimetric method. Quercetin served as the reference standard, and a calibration curve was constructed by plotting absorbance values against known concentrations of quercetin. The extracts were treated with aluminum chloride solution, and absorbance was recorded at 415 nm using a spectrophotometer. The results were expressed as milligrams of Quercetin Equivalents (QE) per gram of extract, with the calibration curve equation obtained through linear regression analysis.

2.9 Qualitative Analysis by GC-MS Method

GC-MS analysis of *S. aromaticum* and *P. nigrum* extracts was performed on a SHIMADZU QP2010 system (Elite-1 capillary column, 30 m × 0.25 mm, 0.25 µm) with helium (99.99%) as carrier gas (1.51 mL/min, 70 eV). A 2 µL sample (split 20:1) was injected, and spectra were recorded from 40-1000 m/z over a 35-min run (oven: 70-300°C). Analysis was conducted at Bio NEST, SPMVV-Women Biotech Incubation Facility, Tirupati, Andhra Pradesh[32].

2.10 Quantitative Analysis by the HPLC Method

High-Performance Liquid ChromatoFigurey (HPLC) was used to quantify major bioactive compounds in the extracts[33]. Eugenol from *S. aromaticum* was quantified using a Shimadzu Prominence HPLC system fitted with an LC-20AT pump, SPD-20A UV-Vis detector, Phenomenex Kinetex C18 column, and a Rheodyne injector. The analysis was carried out with an acetonitrile-methanol mobile phase in a 1:1 ratio, maintained at a flow rate of 1.0 mL/min, and monitored at 281 nm. Piperine in *P. nigrum* was quantified under similar conditions with a mobile phase of acetonitrile:water: acetic acid (60:39.5:0.5 v/v) and detection at 340 nm. Calibration curves prepared with standard eugenol and piperine solute ions were used for quantification. All measurements were performed in triplicate.

2.11 Collection of Microbial Cultures

The oral pathogenic bacterial strains to be examined, *Streptococcus mutans* (ATCC25175), *Porphyromonas gingivalis* (ATCC33277), *Enterococcus faecalis* (ATCC29212), and *Treponema denticola* (ATCC35405), were collected from Himedia and LGC laboratories, India, and cultured on their respective agar media plates.

2.12 Anti-microbial Activity by Well Diffusion Method

The antimicrobial effectiveness of the extracts was tested by the disk diffusion method, using sterile Mueller-Hinton agar plates. Microbial inocula were standardised to match a 0.5 McFarland turbidity, then uniformly spread across each plate to create an even lawn. Sterile 6 mm wells were made by the borer, and each was loaded with 20 µL of extract at a concentration of 50 mg/mL on the inoculated plates. and incubated at 37°C for 24 h. After incubation, zones of inhibition were accurately measured in millimetres using a digital caliper. Chlorhexidine gluconate (0.2%) served as the positive control (PC), and distilled water was included as the negative control (NC)[34].

2.13 Determination of MIC and MBC

The MIC of each extract was determined using the broth dilution method. The dilutions of the extracts, ranging from 0.25 to 100 mg/mL (0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,20, 30, 40, 50 and 100mg/mL), were prepared using a modified dilution method (using finer incremental method below 10 mg/mL for accurate MIC determination and with broader intervals at higher concentrations for screening with different ranges) in Mueller Hinton broth. In 96-well microtiter plates, each well was inoculated with 100 µL of microbial suspension (10^6 CFU/mL) and 100 µL of the respective extract dilution. The plates were incubated at 37°C. The MIC was identified as the lowest concentration of the extract at which no visible microbial growth was observed[35]. The MBC was determined by subculturing samples from wells with no visible growth in the MIC assay onto fresh Mueller-Hinton agar plates. After incubation, the plates were examined for bacterial survival. The MBC was defined as the lowest concentration of extract that resulted in killing at least 99.9% of the initial bacterial population. All assays were performed in triplicate for each bacterial strain to ensure reproducibility.

2.14 Assay for Biofilm Inhibition

The biofilm inhibition assay was conducted in flat-bottom six-well plates. Overnight cultures of *Streptococcus mutans* (ATCC25175), *Porphyromonas gingivalis* (ATCC33277), *Enterococcus faecalis* (ATCC29212), and *Treponema denticola* (ATCC35405) were grown in fresh BHI (Brain Heart Infusion) broth enriched with 5% sucrose and incubated anaerobically at 37°C until reaching mid-log phase (OD600 ≈ 1.0). The bacterial suspensions were diluted 1:7 and added to the wells along with different concentrations of herbal extracts. Chlorhexidine gluconate (0.2%) served as the positive control, distilled water as the negative control, and sterile media without bacteria as the sterility control. Wells containing bacteria but no extract were used as growth controls. The plates were then incubated at 37°C for 24 h. This setup allows evaluation of the herbal extract's ability to inhibit biofilm formation by these oral bacteria under controlled anaerobic conditions[36]. After incubation, bacterial cultures were removed by inverting the six-well plates, and wells were gently washed with distilled water to eliminate planktonic and loosely attached cells. The remaining attached cells were fixed with methanol for 20 min, followed by decantation and air drying. Cells were then stained with 0.1% crystal violet for 20 min, washed three times with distilled water, and air-dried. Biofilm formation was assessed visually as well as absorbance at 590 nm and 570 nm; violet staining at the well bottoms indicated biofilm development, whereas reduced or absent staining indicated biofilm inhibition[37]. Percentage inhibition was calculated by using the equation:

$$\% \text{ Inhibition} = [1 - (\text{Absorbance of the test} / \text{Absorbance of non-treated control})] \times 100$$

2.15 Protein Leakage Assay

Protein leakage from bacterial cells was quantified using the Bradford assay. Bacterial cultures were treated with 25, 50, and 100 µL of different extracts of *S. aromaticum* and *P. nigrum* for 12 h. After treatment, samples were centrifuged at 6000 rpm for 15 min, and the supernatant was collected. For the assay, 200 µL of supernatant was mixed with 800 µL of Bradford reagent and incubated in the dark for 10 min. The optical density was measured at 595 nm, using Bovine Serum Albumin (BSA) as the protein standard[38].

2.16 Cytoplasmic Leakage Assay

DNA leakage was assessed to evaluate the loss of cell membrane integrity. A 10 mL aliquot of a 24-hour bacterial culture was centrifuged at 3500 rpm for 15 min, and the resulting pellets were washed with phosphate buffer (pH = 7). The pellets were then resuspended in 10 mL of the same buffer and incubated with shaking for 24 h. Different concentrations of *S. aromaticum* and *P. nigrum* extracts (25, 50, and 100 µg/mL) were added to the suspension, followed by centrifugation at 3500 rpm for 15 min and then the collected supernatant was analysed at 595 nm using a spectrophotometer[39].

2.17 Statistical Analysis

All experimental data were represented as mean ± standard deviation (SD) of 3 independent replicates (n = 3). The mean was the average of the results obtained from the replicates, and the SD is the measure of the precision and variability of the measurements. Significant differences between treatment groups were determined by one-way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test, with $p<0.05$ as the level of statistical significance.

3. Results

S. aromaticum exhibited (Table 1) a slightly higher ash content (5.86 ± 0.06%) than *P. nigrum* (4.81 ± 0.07%), indicating greater inorganic matter. Pressure-treated extracts from dry heat-processed herbs showed higher concentrations, MTS, and %AEV compared to their decoctions; SaP had 1.967 ± 0.101 mg/ml concentration and 18.03% AEV, while SaD had 1.397 ± 0.031 mg/ml and 13.17% AEV. Similarly, PnP (1.870 ± 0.060 mg/ml; 17.56% AEV) surpassed PnD (1.463 ± 0.102 mg/ml; 13.08% AEV). These results suggest that dry heat-pressure-treated extracts yield higher soluble phytoconstituents than decoctions.

Both *S. aromaticum* and *P. nigrum* decoctions (SaD, PnD) and pressure-treated extracts (SaP, PnP) retained their characteristic colour, pungent odour, and spicy taste (Table 2). The pH values indicated mild acidity, ranging from 4.97 ± 0.12 (SaP) to 5.67 ± 0.068 (PnD), with pressure-treated extracts showing slightly lower pH. Pressure-treated extracts also exhibited higher viscosity (SaP: 1.35 ± 0.01 cp; PnP: 1.26 ± 0.01 cp), density (SaP: 1.10 ± 0.01 g/mL; PnP: 1.09 ± 0.01 g/mL), and optical density (SaP: 0.673 ± 0.001; PnP: 0.587 ± 0.001) compared to decoctions.

Table 1. Ash content of *S. aromaticum* and *P. nigrum* powders, concentration, MTS and %AEV of SaD, SaP, PnD and PnP

Powder	%Ash (Mean±SD)	Extract	Concentration (mg/mL) (Mean±SD)	MTS (gm) (Mean±SD)	% AEV (%w/w)
S. aromaticum	5.86±0.06	SaD	1.397±0.031	0.676±0.015	13.172
		SaP	1.967±0.101	0.940±0.048	18.030
P. nigrum	4.81±0.07	PnD	1.463±0.102	0.678±0.047	13.085
		PnP	1.870±0.060	0.883±0.028	17.561

MTS - Mass of total solids, %AEV - Aqueous extractive value Percentage, SD -Standard deviation

Table 2. Organoleptic and physicochemical characterisation of SaD, SaP, PnD and PnP

Parameters	SaD	SaP	PnD	PnP
Colour	Dark brown	Dark brown	Grayish black	Grayish black
Odour	Pungent	Pungent	Pungent	Pungent
Taste	spicy	spicy	spicy	spicy
pH (Mean±SD)	5.47±0.06	4.97±0.12	5.67±0.06	5.23±0.58
Viscosity(cp) (Mean±SD)	1.24±0.01	1.35±0.01	1.20±0.01	1.26±0.01
Density (Mean±SD)	1.09±0.01	1.10±0.01	1.08±0.01	1.09±0.01
OD_{600} (Mean±SD)	0.573±0.001	0.673±0.001	0.486±0.001	0.587±0.001

OD_{600}-Optical density at 600 nm

Table 3. Stability assessment of SaD, SaP, PnD and PnP at room temperature

Day	Extract	Colour	Odour	Taste	pH (Mean±SD)	Viscosity (cp) (Mean±SD)	Turbidity	OD_{600} (Mean±SD)	Microbial growth
1	SaD	NC	NC	NC	5.47±0.06	1.24±0.01	-	0.573±0.001	-
	SaP	NC	NC	NC	4.97±0.12	1.35±0.01	-	0.673±0.001	-
	PnD	NC	NC	NC	5.67±0.06	1.2±0.01	-	0.486±0.001	-
	PnP	NC	NC	NC	5.23±0.06	1.26±0.01	-	0.587±0.001	-
2	SaD	NC	NC	NC	5.17±0.06	1.25±0.01	-	0.590±0.003	-
	SaP	NC	NC	NC	4.87±0.06	1.35±0.01	-	0.678±0.002	-
	PnD	NC	NC	NC	5.33±0.06	1.21±0.01	-	0.497±0.002	-
	PnP	NC	NC	NC	5.20±0.00	1.26±0.01	-	0.581±0.001	-
3	SaD	C	NC	C	4.93±0.06	1.32±0.01	+	0.836±0.002	+
	SaP	NC	NC	NC	4.83±0.06	1.36±0.01	-	0.757±0.002	-
	PnD	C	NC	C	5.07±0.06	1.28±0.01	+	0.745±0.002	+
	PnP	NC	NC	NC	5.13±0.06	1.27±0.01	-	0.654±0.001	-

NC - Not changed, C - Changed, "+" - Present, "-" - Absent

Table 4. Stability assessment of SaD, SaP, PnD and PnP at 4°C

Day	Extract	Colour	Odour	Taste	pH (Mean±SD)	Viscosity (cp) (Mean±SD)	Turbidity	OD_{600} (Mean±SD)	Microbial growth
7	SaD	NC	NC	NC	5.33±0.06	1.24±0.01	-	0.582±0.001	-
	SaP	NC	NC	NC	4.87±0.06	1.36±0.01	-	0.679±0.001	-
	PnD	NC	NC	NC	5.33±0.06	1.21±0.01	-	0.491±0.000	-
	PnP	NC	NC	NC	5.17±0.06	1.26±0.01	-	0.591±0.002	-
14	SaD	NC	NC	NC	5.43±0.06	1.26±0.01	-	0.595±0.000	-
	SaP	NC	NC	NC	4.77±0.06	1.37±0.01	-	0.683±0.002	-
	PnD	NC	NC	NC	5.43±0.06	1.23±0.01	-	0.504±0.001	-
	PnP	NC	NC	NC	5.17±0.06	1.27±0.01	-	0.598±0.001	-
21	SaD	NC	NC	NC	5.33±0.06	1.27±0.01	-	0.614±0.001	-
	SaP	NC	NC	NC	4.87±0.06	1.38±0.01	-	0.694±0.002	-
	PnD	NC	NC	NC	5.33±0.06	1.28±0.01	-	0.537±0.000	-
	PnP	NC	NC	NC	5.27±0.06	1.29±0.01	-	0.614±0.003	-
28	SaD	NC	NC	NC	5.33±0.06	1.30±0.01	+	0.653±0.001	+
	SaP	NC	NC	NC	4.87±0.06	1.38±0.01	-	0.709±0.003	-
	PnD	NC	NC	NC	5.43±0.06	1.39±0.01	+	0.582±0.002	+
	PnP	NC	NC	NC	5.27±0.06	1.32±0.01	-	0.629±0.002	-

NC - Not changed, C - Changed, "+" - Present, "-" - Absent

Stability assessment of all *S. aromaticum* and *P. nigrum* extracts was done at room temperature over 3 days. Observations of colour, odour, taste, pH, viscosity, turbidity, optical density (OD_{600}), and microbial growth indicated that pressure-treated extracts remained more stable than decoctions under ambient conditions (Table 3).

Stability of *S. aromaticum* and *P. nigrum* extracts (SaD, SaP, PnD, PnP) was also assessed at 4°C over 28 days. Change in colour, odour or taste was not observed throughout the study, confirming sensory stability. SaP kept a constant acidic pH of 4.77-4.87 without any significant change over time, indicating

Table 5. Preliminary phytochemical analysis of SaD, SaP, PnD and PnP

Phytoconstituent	Test Name	*S. aromaticum*		*P. nigrum*	
		SaD	SaP	PnD	PnP
Alkaloids	Dragendroff's	+	+	+	+
	Mayer's	+	+	+	+
Flavanoids	Shinoda	+	+	+	+
	Alkaline reagent	+	+	+	+
Tannins	Ferric chloride	+	+	+	+
	Gelatine	+	+	+	+
Saponins	Foam	+	+	+	+
Phenolic compounds	Ferric chloride	+	+	+	+
	Lead acetate	+	+	+	+

"+" - Present, "-" - Absent

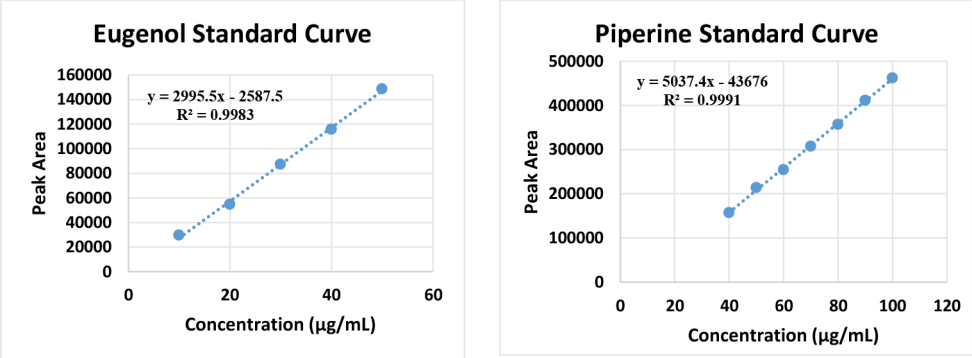


Figure 1. Standard curves of eugenol and piperine.

high stability of bioactive components in the SaP. PnP was also stable, and pH was not changed much over the course of the experiment, ranging from 5.17 ±0.06 on Day 7 to 5.27 ± 0.06 on Day 28. SaP exhibited the highest and the most stable viscosity, which barely increased by 1.36 ±0.01 cP (Day 7) to 1.38±0.01 cP (Day 28). Similarly, PnP showed a controlled increase from 1.26 ±0.01 cp to 1.32 ±0.01 cp in a narrow stable range. Optical density (OD600) had a slight increase over time, indicating little development of turbidity, especially in decoctions (SaD = 0.653 (±0.001); PnD = 0.582 (±0.002) at Day 28). Microbial growth was only observed in decoctions after 28 days, while SaP and PnP were stable till 28 days (Table 4).

All four extracts, SaD, SaP, PnD, and PnP, tested positive for alkaloids, flavonoids, tannins, saponins, and phenolic compounds, indicating a broad spectrum of phytoconstituents contributing to their potential bioactivity (Table 5).

Dry heat-pressure treated extracts contained higher total phenolics (SaP: 65.94 ± 0.47 mg GAE/g; PnP: 53.12 ± 0.56 mg GAE/g) and flavonoids (SaP: 125.23 ± 0.61 mg QE/g; PnP: 114.54 ± 0.56 mg QE/g) than their decoctions. Similarly, bioactive compounds were higher in pressure-treated extracts, with eugenol in *S. aromaticum* rising from 14.15 ± 0.004 to 22.40 ± 0.003 mg/g, and piperine in *P. nigrum* from 17.22 ± 0.25 to 22.28 ± 0.22 mg/g. All increases were statistically significant ($p<0.05$), indicating enhanced phytochemical yield in pressure-treated extracts (Figures 1 and 2, Table 6).

GC-MS analysis of SaD and SaP extracts (Figure 3, Table 7) reported that both extracts had similar compounds composed of eugenol, with a major constituent of 38.26% of SaD and 41.41% of SaP. Other important compounds were caryophyllene (10.28% in SaD; 11.43% in SaP), phenol compounds, and eugenyl acetate, whereas minor compounds such as humulene, chavicol and fatty acid esters were also observed.

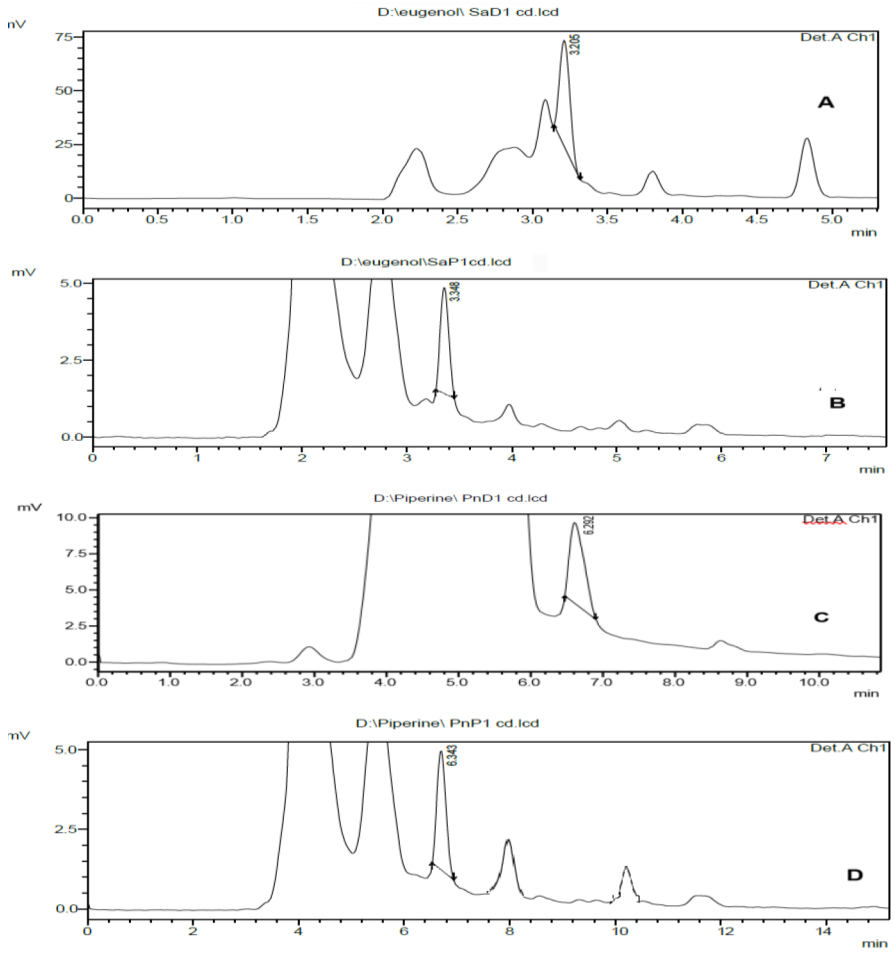


Figure 2. HPLC chromatograms of SaD(A), SaP(B), PnD(C) and PnP(D).

Table 6. Quantitative phytochemical analysis of SaD, SaP, PnD and PnP

Compound	Extract	*S. aromaticum*	*P. nigrum*	pvalue
Total Phenolic Content (mg GAE/g)	D	47.72±0.47	44.40±0.96	<0.05
	P	65.94±0.53	53.12±0.56	<0.05
Total flavonoid Content (mg QE/g)	D	84.88±0.51	76.00±0.41	<0.05
	P	125.23±0.61	114.54±0.56	<0.05
Eugenol content (mg/g)	D	14.15±0.004	-	<0.01
	P	22.40±0.003	-	
Piperine Content (mg/g)	D	-	17.22±0.25	<0.01
	P	-	22.28±0.22	

D - Decoction; P - Pressure-treated aqueous extract

Overall, SaP showed slightly higher relative abundances for important bioactive compounds, indicating that the pressure-treated extracts may be more effective at increasing the concentration of phytochemicals than the decoction.

GC-MS analysis of PnD and PnP extracts (Figure 3, Table 8) reported that both extracts contained similar compounds, with palmitic acid (16.52% in PnD; 16.98% in PnP) and stearic acid (15.81% in PnD; 15.77% in PnP) as the major constituents. Other notable components

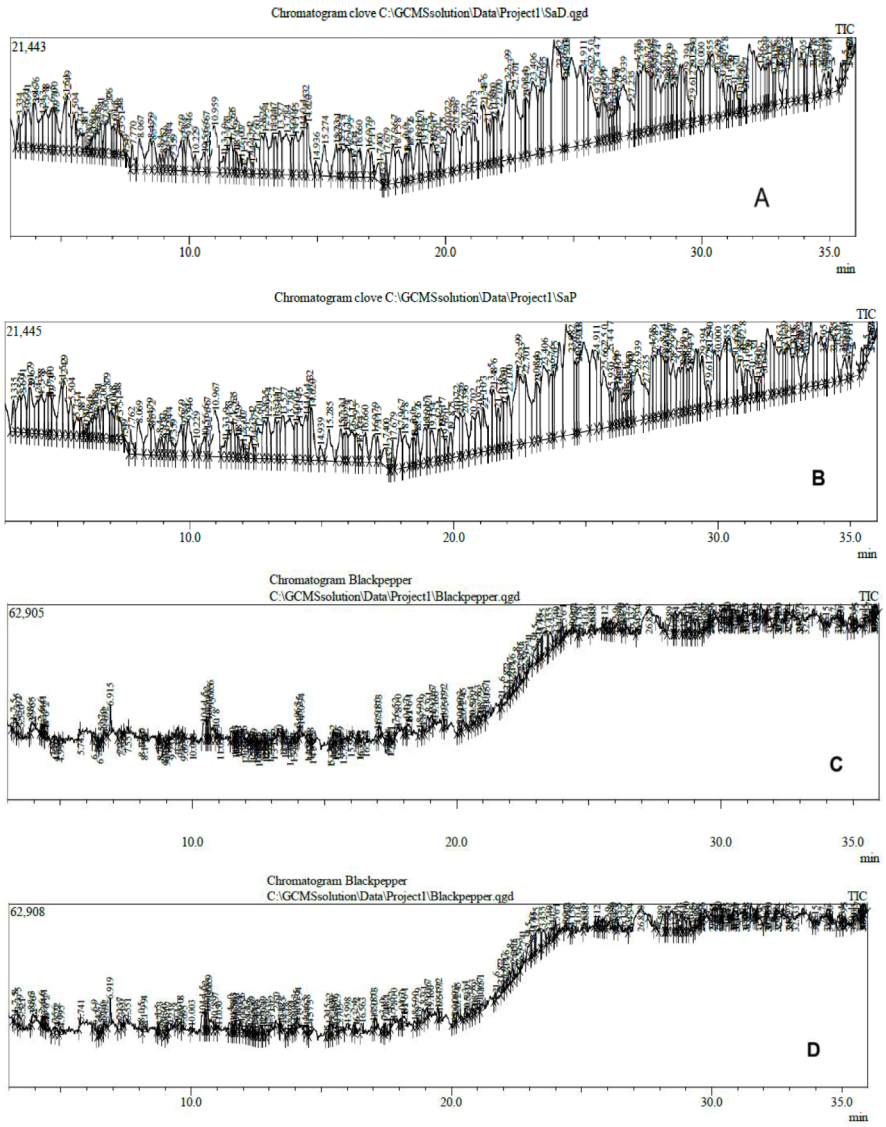


Figure 3. GC-MS Chromatograms of SaD(A), SaP(B), PnD(C) and PnP(D).

included 2-ethylbenzotriazole, methyl linoleate, methyl linolelaidate, and petroselinic acid, while minor constituents included phthalate esters and long-chain fatty acid derivatives. Overall, the PnP showed slightly higher relative abundances for several key compounds, indicating that pressure-treated extracts may enhance the concentration of bioactive constituents compared to decoction.

SaP and PnP always had larger zones of inhibition than their decoction forms. SaP showed an inhibition zone of 16.33 ± 0.58 mm against *S. mutans* and 15.00 ± 1.00 mm against *P. gingivalis,* while PnP showed 14.67 ± 0.58 mm and 12.67 ± 1.15 mm, respectively (Table 9). Decoctions had a relatively lower activity, and SaD and PnD led to smaller inhibition zones for all the tested pathogens. These results show that SaP and PnP have a greater antimicrobial efficacy against oral pathogens than SaD and PnD.

SaP and PnP showed lower MIC values than decoctions, which indicates stronger antimicrobial potency. SaP demonstrated the best activity with MIC values of 6.67±0.58 to 10.00±0.00 mg/mL (Table 10) in the tested pathogens, whereas SaD and PnD required higher concentrations for inhibition. SaP showed MBCs of 8.00 ± 0.00 mg/mL against *S. mutans* and 10.00 ± 0.00 mg/mL against *T. denticola*, while SaD required higher concentrations (9.67-26.67 mg/mL) for bactericidal effect. PnP also performed better than PnD, with MBCs

Table 7. List of identified compounds in SaD and SaP by GC-MS analysis

			SaD		SaP	
No.	**Compound Name**	**Molecular formula**	**RT (min)**	**Area (%)**	**RT (min)**	**Area (%)**
1	Phenol, 4-(2-propenyl)-	$C_9H_{10}O$	7.853	0.36	7.655	0.41
2	Benzaldehyde	C_7H_6O	7.916	0.49	7.819	0.56
3	2-Propenal, 3-phenyl-	C_9H_8O	8.028	0.32	8.127	0.34
4	Eugenol	$C_{10}H_{12}O_2$	8.432	38.26	8.229	41.41
5	Bicyclo[3.1.1]heptan-3-ol	$C_{10}H_{16}O$	8.737	1.09	8.634	1.12
6	2-Nonanone	$C_9H_{18}O$	9.121	0.33	9.228	0.32
7	1,2,3-Benzenetriol	$C_6H_6O_3$	9.254	0.58	9.164	0.67
8	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	9.569	0.12	9.639	0.16
9	Naphtho[2,3-c]furan-1,3-dione	$C_{12}H_6O_3$	9.595	0.11	9.655	0.11
10	Caryophyllene	$C_{15}H_{24}$	10.253	10.28	10.136	11.43
11	Humulene	$C_{15}H_{24}$	10.446	2.72	10.384	2.74
12	Phenol, 2-methoxy-4-(2-propenyl)-acetate	$C_{12}H_{14}O_3$	10.793	7.17	10.648	7.22
13	Caryophyllene oxide	$C_{15}H_{24}O$	11.588	0.71	11.449	0.77
14	2,3,4-Trimethoxyacetophenone	$C_{11}H_{14}O_4$	12.346	0.65	12.338	0.69
15	Eugenyl acetate	$C_{12}H_{14}O_3$	12.843	2.54	12.614	3.21
16	Acetic acid, phenylmethyl ester	$C_9H_{10}O_2$	13.142	0.52	13.048	0.55
17	Methyl salicylate	$C_8H_8O_3$	13.744	0.16	13.693	0.18
18	Chavicol	$C_{10}H_{14}O$	14.321	0.35	15.111	0.37
19	1-Propyl-3-(propen-1-yl)adamantane	$C_{16}H_{26}$	15.074	1.53	14.678	1.51
20	Decanal	$C_{10}H_{20}O$	16.384	0.75	16.276	0.77
21	n-Hexadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	17.027	2.15	16.927	2.11

of 9.33-13.33 mg/mL compared to 20.00-26.67 mg/mL in decoctions. These results indicated that dry heat-pressure-treated extracts have enhanced bactericidal efficacy against oral pathogens.

SaP and PnP demonstrated higher inhibition compared to decoctions (Table 11). At 590 nm, SaP inhibited biofilm formation by 77.08 ± 0.31% and PnP by 66.90 ± 0.31%, while SaD and PnD showed 65.50 ± 0.42% and 55.28 ± 0.67%, respectively. Similar trends were observed at 490 nm. These results indicate that pressure-treated extracts are more effective in preventing biofilm formation than decoction forms.

Table 12 presents protein leakage from oral pathogens in the presence of *S. aromaticum* and *P. nigrum* extracts at different volumes (25, 50, 100 µl). Protein leakage increased with extract concentration for all pathogens. SaP and PnP induced higher leakage than decoctions, indicating greater membrane disruption. For instance, against *S. mutans* at 100 µl, SaP caused an absorbance of 0.572 ± 0.001, PnP 0.552 ± 0.001, whereas SaD and PnD showed 0.527 ± 0.001 and 0.518 ± 0.001, respectively. Similar trends were observed across *E. faecalis*, *P. gingivalis*, and *T. denticola*, suggesting that pressure-treated extracts have stronger bactericidal effects through membrane damage.

Table 13 shows cytoplasmic leakage from oral pathogens in response to *S. aromaticum* and *P. nigrum* extracts at 25, 50, and 100 µL. Leakage was increased with increased extract volume for all pathogens. SaP and PnP caused higher leakage than decoctions, indicating stronger membrane disruption against oral pathogens. *S. mutans* at 100 µL, SaP has shown 0.593 ± 0.001 absorbance and PnP 0.583 ± 0.001, compared to 0.527 ± 0.001 and 0.525 ± 0.001 for SaD and PnD. Similar patterns were noticed for *E. faecalis, P. gingivalis*, and *T. denticola*, implying enhanced bactericidal activity of pressure-treated extracts through cytoplasmic content release.

Table 8. List of identified Compounds in PnD and PnP by GC-MS analysis

			PnD		PnP	
No	**Component**	**Molecular formula**	**RT (min)**	**Area %**	**RT (min)**	**Area %**
1	2-Ethylbenzotriazole	$C_8H_9N_3$	4.66	10.17	4.546	10.21
2	Caryophyllen	$C_{15}H_{24}$	9.526	1.56	9.412	1.6
3	Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-	$C_{15}H_{22}O$	9.526	1.51	9.432	1.63
4	2,4-di-tert-butylphenol	$C_{14}H_{22}O$	9.959	1.94	9.845	1.98
5	Methyl palmitate	$C_{17}H_{34}O_2$	14.468	2.57	14.356	2.73
6	Methyl 14-methylpentadecanoate	$C_{17}H_{34}O_2$	14.471	2.52	14.356	2.73
7	Dibutyl phthalate	$C_{16}H_{22}O_4$	14.812	6.01	14.695	6.13
8	Butyl isobutyl phthalate	$C_{16}H_{22}O_4$	14.803	6.04	14.689	6.16
9	Palmitic acid	$C_{16}H_{32}O_2$	14.947	16.52	14.833	16.98
10	Methyl linoleate	$C_{18}H_{32}O_2$	16.058	8.52	15.944	8.58
11	Methyl linolelaidate	$C_{19}H_{34}O_2$	16.098	8.56	15.891	8.64
12	9-cis,11-trans-Octadecadienoate	$C_{18}H_{31}O_2$	16.068	8.42	15.934	8.49
13	9-Octadecenoic acid (Z), methyl ester	$C_{19}H_{36}O_2$	16.114	4.26	16	4.22
14	Petroselinic acid (6-Octadecenoic acid)	$C_{16}H_{34}O_2$	16.514	9.54	16.424	9.65
15	(9E,12Z)-linoleic acid	$C_{18}H_{32}O_2$	16.514	9.51	16.489	9.65
16	Stearic acid	$C_{18}H_{36}O_2$	16.781	15.81	16.667	15.77
17	Carbonic acid, decyl undecyl ester	$C_{22}H_{44}O_3$	18.225	0.7	18.111	0.66
18	Eicosane	$C_{20}H_{42}$	19.569	0.53	19.455	0.49
19	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	19.836	1.844	19.722	1.923
20	Phthalic acid, dodecyl 2-ethylhexyl ester	$C_{28}H_{46}O_4$	19.831	1.846	19.727	1.935
21	Phthalic acid, hexadecyl 4-methylhept-3-yl ester	$C_{32}H_{54}O_4$	19.836	1.84	19.729	1.79

Table 9. Antimicrobial activity of SaD, SaP, PnD and PnP against various oral pathogens (Mean ± Standard deviation of Zone of inhibition)

Oral Pathogen	NC	PC (mm)	SaD (mm)	SaP (mm)	pvalue	PnD (mm)	PnP (mm)	pvalue
S. mutans	NZ	17.67±1.53	12.00±1.00	16.33±0.58	0.003	9.33±0.58	14.67±0.58	0.000
E. faecalis	NZ	16.67±1.53	12.67±1.15	16.00±1.00	0.019	10.33±0.58	14.33±1.15	0.006
P. gingivalis	NZ	16.00±1.73	10.33±1.15	15.00±1.00	0.006	6.67±0.58	12.67±1.15	0.001
T. denticola	NZ	15.00±1.00	9.67±2.08	14.33±0.58	0.020	7.33±1.15	12.00±1.00	0.006

C - Negative control (Distilled water), PC - Positive control (Chlorhexidine gluconate - 0.2%), NZ - No Zone

4. Discussion

Herbal extraction procedures have evolved to become safer, more efficient, and eco-friendly, emphasising the replacement of toxic solvents with non-toxic, sustainable alternatives in line with green chemistry principles[40]. Although research seeks higher yield and efficiency, water remains the most important solvent, and this study compared pressure-treated aqueous extracts with traditional decoctions to develop safe, cost-effective, high-quality natural oral care formulations.

Clove showed slightly higher ash content than black pepper, indicating more inorganic residue, though both remained within acceptable limits[41,42]. SaP and PnP's higher yields, concentration and extractive

Table 10. MIC and MBC of SaD, SaP, PnD and PnP (mg/mL) against various oral pathogens (Mean ± Standard deviation)

Oral Pathogen		NC	PC (mg/mL)	SaD (mg/mL)	SaP (mg/mL)	pvalue	PnD (mg/mL)	PnP (mg/mL)	pvalue
S. mutans	M	-	1.00±0.00	8.67±0.58	6.67±0.58	0.013	10.00±0.00	8.00±1.00	0.026
E. faecalis	I	-	1.00±0.00	9.33±0.58	7.67±0.58	0.024	9.33±0.58	7.33±0.58	0.013
P. gingivalis	C	-	3.00±0.00	9.67±0.58	7.67±0.58	0.013	10.00±0.00	8.67±0.58	0.016
T. denticola		-	3.00±0.00	9.67±0.58	8.00±0.00	0.007	9.67±0.58	8.33±0.58	0.047
S. mutans	M	-	2.00±0.00	9.67±0.58	8.00±0.00	0.01	20.00±0.00	9.33±0.58	0.00
E. faecalis	B	-	2.00±0.00	10.00±0.00	8.67±0.58	0.02	20.00±0.00	9.67±0.58	0.00
P. gingivalis	C	-	3.67±0.58	23.33±5.77	10.00±0.00	0.02	26.67±5.77	13.33±5.77	0.05
T. denticola		-	4.00±0.00	26.67±5.77	10.00±0.00	0.01	23.33±5.77	10.00±0.00	0.02

NC - Negative control (Distilled water), PC - Positive control (Chlorhexidine gluconate - 0.2%)

Table 11. Percentage of biofilm inhibition with SaD, SaP, PnD and PnP (Mean ± Standard deviation)

Wavelength (nm)	NC	PC	SaD	SaP	pvalue	PnD	PnP	p-value
590	-	77.00±0.64	65.50±0.42	77.08±0.31	<0.01	55.28±0.67	66.90±0.31	<0.01
570	-	74.45±0.19	60.25±0.11	72.49±0.39	<0.01	54.70±0.19	60.44±0.26	<0.01

NC - Negative control (Distilled water), PC - Positive control (Chlorhexidine gluconate - 0.2%)

Table 12. Protein leakage assay of SaD, SaP, PnD and PnP against various oral pathogens (Mean ± Standard deviation)

		Absorbance at 595nm				
Oral Pathogen	Volume of extract (µL)	PC	SaD	SaP	PnD	PnP
S. mutans	25	0.092±0.001	0.132±0.001	0.161±0.002	0.129±0.001	0.137±0.000
	50	0.204±0.001	0.291±0.002	0.320±0.001	0.288±0.001	0.297±0.001
	100	0.412±0.001	0.527±0.001	0.572±0.001	0.518±0.001	0.552±0.001
E. faecalis	25	0.095±0.001	0.133±0.002	0.158±0.000	0.135±0.001	0.148±0.001
	50	0.206±0.001	0.289±0.002	0.322±0.001	0.291±0.001	0.302±0.001
	100	0.411±0.001	0.532±0.001	0.564±0.005	0.520±0.001	0.547±0.001
P. gingivalis	25	0.089±0.000	0.116±0.001	0.143±0.001	0.108±0.001	0.117±0.001
	50	0.193±0.001	0.264±0.001	0.317±0.002	0.252±0.001	0.264±0.001
	100	0.404±0.001	0.485±0.001	0.575±0.001	0.481±0.001	0.548±0.001
T. denticola	25	0.087±0.001	0.122±0.001	0.137±0.001	0.117±0.002	0.127±0.001
	50	0.193±0.001	0.257±0.001	0.306±0.001	0.245±0.001	0.268±0.001
	100	0.402±0.001	0.474±0.001	0.556±0.001	0.468±0.001	0.534±0.001

PC - Positive control (Chlorhexidine gluconate - 0.2%)

values confirm that pressure-treated extraction more effectively releases phytochemicals by enhancing cell wall disruption and solvent penetration. Organoleptic and physicochemical analyses showed that both decoctions and pressure-treated extracts retained characteristic traits, but slightly lower pH but higher viscosity, density, and optical density were observed in pressure-treated extracts, reflecting greater solubilisation of bioactives. These results support previous findings on increased yield of phytochemicals and consistency of the extracts with the use of pressure assisted extraction[43-45].

Table 13. Cytoplasmic leakage assay of SaD, SaP, PnD and PnP against various oral pathogens (Mean ± Standard deviation)

		Absorbance at 595nm				
Oral Pathogen	**Volume of extract (µL)**	**PC**	**SaD**	**SaP**	**PnD**	**PnP**
S. mutans	25	0.123±0.001	0.132±0.001	0.176±0.002	0.163±0.002	0.186±0.001
	50	0.245±0.001	0.291±0.002	0.344±0.002	0.303±0.002	0.317±0.001
	100	0.483±0.001	0.527±0.001	0.593±0.001	0.525±0.001	0.583±0.001
E. faecalis	25	0.119±0.002	0.133±0.002	0.168±0.001	0.154±0.002	0.168±0.002
	50	0.227±0.001	0.289±0.002	0.342±0.002	0.325±0.001	0.332±0.001
	100	0.441±0.001	0.532±0.001	0.587±0.002	0.543±0.002	0.566±0.001
P. gingivalis	25	0.106±0.001	0.116±0.001	0.164±0.001	0.128±0.001	0.137±0.001
	50	0.215±0.001	0.264±0.001	0.335±0.004	0.275±0.001	0.284±0.001
	100	0.425±0.001	0.485±0.001	0.596±0.002	0.502±0.001	0.548±0.001
T. denticola	25	0.108±0.001	0.122±0.001	0.158±0.002	0.132±0.002	0.147±0.001
	50	0.222±0.002	0.257±0.001	0.329±0.002	0.264±0.001	0.290±0.003
	100	0.402±0.001	0.474±0.001	0.577±0.001	0.485±0.002	0.555±0.001

PC - Positive control (Chlorhexidine gluconate - 0.2%)

Pressure-treated extracts stayed stable and microbe-free far longer than decoctions, showing clearly better shelf life and physical stability at both room temperature and refrigeration. These findings are in agreement with previous works that reported the better chemical and microbiological stability of herbal and phenolic-rich matrices during pressure-based extraction methods, such as high pressure or hydrostatic processing, inducing efficient extractions of bioactive compounds and a decrease in water activity[43-45]. Therefore, pressure-treated extracts of *S. aromaticum* and *P. nigrum* show a better physicochemical and microbiological stability compared to the use of conventional decoctions, making them more suitable for short-term storage and formulation use. While the current stability assessment provides valuable insights for initial formulation, extended stability studies under ICH guidelines are recommended.

Both decoctions and pressure-treated extracts contained similar key phytochemicals, but pressurisation better preserved and enhanced bioactive release, supporting their strong antimicrobial potential. Dry heat treatment of clove and black pepper before extraction enhances the release of certain bioactive compounds and improves their antioxidant potential in the treated samples[26].

The quantitative phytochemical evaluation showed a significant increase in total phenolics, flavonoids and some of the most important bioactive compounds in the pressure-treated extracts (SaP and PnP) than the decoctions (SaD and PnD). Likewise, concentrations of eugenol and piperine were significantly enhanced in pressure-treated samples, indicating improved extraction efficiency and compound stability in optimised pressure conditions. Pressure-assisted extraction at 121°C for 20 min likely enhances bioactive recovery by rapidly disrupting plant cell walls while minimising prolonged thermal exposure. In contrast, conventional decoction involves extended boiling at 100°C, which may promote oxidative degradation or hydrolysis of thermolabile compounds such as eugenol and piperine[43-45]. The shorter, controlled thermal profile of autoclave-based extraction thus better preserves labile phytoconstituents, aligning with green extraction principles.

GC-MS analysis showed eugenol as the major compound in clove, higher in SaP with caryophyllene, eugenyl acetate, and phenolics than SaD, while black pepper extracts (PnP) had slightly higher levels of palmitic and stearic acids and other bioactives, confirming that pressure-treated extraction enhances yield and recovery of key phytochemicals. These results are in agreement with previous results on enhanced pressure-treated extraction of fatty acids and phenolic compounds from spice extracts[46-48].

The superior antimicrobial efficacy of pressure-treated extracts can be explained by the increased concentrations of bioactive compounds such as eugenol, caryophyllene, palmitic and linoleic acids, which are known for disrupting microbial membranes[49]. The decoction, on the other hand, may undergo the partial loss of thermolabile constituents. These findings are in agreement with earlier studies of the potent antibacterial effects of *S. aromaticum* and *P. nigrum* against oral microorganisms[50] and indicate that pressure extraction enhances the retention of phytochemicals and their antimicrobial properties of herbal preparations for oral health applications.

The MIC results demonstrated that pressure-treated extracts (SaP and PnP) of *S. aromaticum* and *P. nigrum* exhibited superior antimicrobial efficacy compared to their decoctions. SaP displayed the lowest MIC values (6.67-10.00 mg/mL), indicating a higher potency against *S. mutans, E. faecalis, P. gingivalis,* and *T. denticola*. The enhanced activity may be attributed to the increased concentrations of active phytoconstituents such as eugenol, caryophyllene, and phenolic compounds, which exert strong membrane-disruptive and enzyme inhibitory effects on pathogens[51,52]. In contrast, the higher MICs observed in decoctions suggest reduced bioavailability of thermolabile components. These findings are consistent with earlier studies reporting improved antibacterial performance of pressure-treated herbal extracts, emphasising that the pressurisation process enhances phytochemical yield and thereby strengthens antimicrobial potency against oral microorganisms[53].

The results of the MBC confirm the better bactericidal activity of the extracts obtained by pressure than the decoctions. SaP and PnP always required lower concentrations to elicit bacterial killing, especially against *S. mutans* and other oral pathogens, whereas decoctions exhibited lesser activity and also higher values for the MBC. This enhanced effectiveness corresponds with the higher phytochemical content of pressure-treated extracts; this indicates that the pressurisation improves the extraction and availability of bactericidal compounds in *S. aromaticum* and *P. nigrum*.

One of the previous studies suggested that eugenol has the potential to inhibit dental caries development with the inhibitory effects on the *S. mutans* activities like production of acid, adherence and water-insoluble glucan synthesis[54]. In the present study, the biofilm inhibition results demonstrated the increased antimicrobial potential of pressure-treated extracts in comparison to their decoction counterparts. At both 590nm and 490nm, SaP and PnP showed markedly higher inhibitory activity against biofilm formation. SaP showed the best inhibition (77.08 ± 0.31% at 590 nm), followed by PnP (66.90 ±0.31%), whereas the decoctions SaD and PnD had relatively low inhibition percentages.

The superior activity of pressure-treated extracts is likely due to higher levels of phenolics, flavonoids, and bioactives like eugenol and piperine, while decoctions show lower efficacy from partial heat-induced degradation of these compounds. These findings indicated that pressure-treated extracts of clove and black pepper exhibit stronger antibiofilm and membrane-disruptive effects on oral pathogens than decoctions, highlighting their potential in oral hygiene formulations.

SaP and PnP caused markedly higher protein and cytoplasmic leakage than decoctions, showing stronger membrane-disrupting action. These effects were consistent with earlier reports that eugenol and piperine-rich extracts disturb phospholipid bilayers of bacterial membranes, increasing membrane permeability and stimulating leakage of proteins[55,56], and confirmed that the membrane disruption mechanism of both spices, with the pressure-treated extracts showing greater bactericidal potential due to enhanced extraction and increased concentrations of active phytochemicals.

The study showed that pressure-treated clove and black pepper extracts had much stronger antimicrobial, antibiofilm, and membrane-disruptive effects than decoctions, making them promising natural oral care agents.

While in this study, promising antimicrobial efficacy has been demonstrated, the safety profile of the extracts warrants consideration. The safety data for eugenol and piperine, the principal bioactives in SaP and PnP, have been established at concentrations comparable to those used herein (eugenol: ≤0.5% w/v in oral rinses; piperine: ≤10 µg/mL, showing no cytotoxicity in oral keratinocytes)[57,58]. Nevertheless, dedicated cytotoxicity assays and acute oral toxicity studies are recommended prior to clinical formulation development.

5. Conclusion

This study demonstrated that pressure-treated aqueous extracts with dry heat-treated herbs outperform decoctions prepared from non-heat-treated herbs, offering richer phytochemicals, better stability, and

stronger antimicrobial and antibiofilm effects essential for preventing oral infections. Pressure-treated aqueous extraction with dry heated herbs is efficient, eco-friendly, and produces extracts with superior antimicrobial potential, supporting their use in safe, natural oral care formulations. Further formulation and clinical studies are suggested for practical application.

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Author Contributions

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