Spittlebugs biofoam: excretory products chemistry, and its pesticidal activity

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ABSTRACT

Poophilus costalis (Aphrophoridae), is known for producing a protective foam during its nymphal stage. This study investigates the chemical and biological properties of the biofoam produced by *P. costalis* nymphs. Stability of the foam decreased significantly at temperatures above 50°C. For the first time, metabolic waste products such as urea, uric acid, and creatinine were identified in the biofoam. Insecticidal activity of the biofoam was assessed using a choice test against three cotton pests: *Phenacoccus solenopsis*, *Dysdercus koenigii*, and *Aphis craccivora*. The froth exhibited repellent properties against *D. koenigii* and *A. craccivora*, while *P. solenopsis* showed attraction at all concentrations except 0.05%. Antibacterial activity was tested against three phytopathogens (*Xanthomonas malvacearum* and *Xanthomonas citri*, *Pseudomonas aeruginosa*), plant growth promotor (*Proteus vulgaris*) and four microbes including G^+ (*Bacillus subtilis*, *Enterococcus faecalis*) and G^- (*Escherichia coli*, *Klebsiella pneumoniae*). The froth alone and in combination with gallic or tannic acid exhibited significant, dose-dependent inhibition activity. Notably, *E. faecalis* and *E. coli* showed the highest growth suppression. Gallic acid combinations enhanced the inhibition of *E. faecalis* and *X. malvacearum*, while tannic acid was more effective against *X. citri*. These findings suggest that *P. costalis* froth has promising potential as a natural antimicrobial agent against both human and plant pathogens.

Keywords: Gallic acid, Tannic acid, Antibacterial activity, *X. malvacearum, X. citri, B. subtilis and, E. aerogenes, P. aeruginosa, K. pneumoniae*, and *P. vulgaris.*

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INTRODUCTION

Insects have evolved a multitude of adaptive strategies to protect themselves from environmental stressors, predators, and pathogens. Among them, the xylophagous spittlebug *Poophilus costalis* Walker, 1851 (Hemiptera: Aphrophoridae) demonstrates a remarkable form of biochemical defense during its nymphal stage. The insect produces a foamy secretion often referred to as biofoam or froth which envelops the entire body surface of the nymph. This froth acts as a barrier against desiccation, temperature extremes, and predation (Del Campo *et al.,* 2011; Tonelli *et al.,* 2019; Sahayaraj *et al.,* 2021; Sahayaraj *et al.,* 2025). More notably, under natural field conditions, the froth remains consistently free of mold or fungal infestation, its suggesting essential antimicrobial properties (Chang *et al.,* 2019a).

Biochemical investigations have revealed that the presence of carbohydrates, proteins, amino acids, enzymes, and octodecanoic acid and also confirms the C18 fatty acid, another essential fatty acid, palmitic acid (C16), has been reported in spittlebug foam (Tonelli *et al.,* 2019; Sahayaraj *et al.,* 2021). Its exhibits antifungal activity, particularly against *Fusarium oxysporum* f. sp. *pisi*, Snyder and Hansen. (Hypocreales: Nectriaceae) a significant phytopathogen. The froth contains active hydrolytic enzymes, including β-N-acetylglucosaminidase, chitobiosidase, and endochitinase, with respective enzymatic activities (Chang *et al.,* 2019a). These chitinases, likely sourced from the insect's Malpighian tubules and

possibly supplemented by the host plant *Commelina diffusa* Burman F., (Commelinales: Commelinaceae), appear to degrade fungal cell walls composed of chitin, thus inhibiting their growth. Furthermore, the antagonistic bacterium *Stenotrophomonas maltophilia* Hugh, (Xanthomonadales: Xanthomonadaceae) and *Delftia tsuruhatensis* Shigematsu (Burkholderiales: Comamonadaceae) isolated from the froth of the spittlebug *P. costalis* showed antifungal activity against the phytopathogenic fungi *F. oxysporum* f.sp. *pisi* and *Colletotrichum gloeosporioides* Penz. (Glomerellales: Glomerellaceae) (Chang *et al.,* 2019b). *Xanthomonas axonopodis pv. malvacearum* E.F. Smith, (Xanthomonadales: Xanthomonadaceae) causes bacterial blightin cotton plants (Akello *et al.,* 2002) and is currently managed with chemicals (Pathak and Shailesh, 2006). However, spittlebug foam is considered a novel material for managing this pathogen. These observations suggest a synergistic triad of defense insect-derived enzymes, plant-derived inputs, and microbial symbiotic.

Pseudomonas aeruginosa is regarded as a biological control agent against *F. oxysporum* (Thakker *et al.,* 2025) and a phytopathogen (Elrod and Braun, 1942). *Bacillus subtilis* (Hashem *et al.,* 2019) and *Proteus vulgaris* (Yu and Lee, 2013) are regarded as rhizobacteria that stimulate plant growth. Prickypear and fieldpoppy flowers were frequently associated with Enterococci when wild flowers were tested for *Enterococcus faecalis* and *Enterococcus faecium* (Sánchez Valenzuela *et al.,* 2012). According to plant species and tissue type, *Escherichia coli* also survives in plants (Merget *et al.,* 2019). *Klebsiella pneumoniae* is linked to plant growth and encourages the production of luster roots (Zhang *et al.,* 2023; Kumar *et al.,* 2021).

Parallel to these biological defenses, polyphenolic compounds such as tannic acid and gallic acid have collected scientific attention for their antimicrobial potential. Tannic acid (TA), a high-molecular-weight polyphenol composed of gallic acid units bound to a central glucose, is abundantly found in various plant species (Deshpande *et al.,* 1984; Wall *et al.,* 1996; Kardel *et al.,* 2013; Iqbal and Poór, 2024), seaweeds (Fauzi *et al.,* 2018; Petchidurai *et al.,* 2019), wines, and teas (Hoff and Singleton, 1977; Singleton, 1992; Tinkílíç and Uyaník, 2001). TA has demonstrated antibacterial, antifungal, antioxidant, and antiviral activities, and its mechanism of antimicrobial action includes iron chelation, inhibition of bacterial adhesion, and disruption of cell membranes (Chung *et al.,* 1998; Uyama, 2007; Belhaoues *et al.,* 2020).

Likewise, gallic acid (GA), a naturally occurring trihydroxybenzoic acid found in numerous fruits (Boston *et al.,* 2019), herbs (Borde *et al.,* 2011; Harwansh *et al.,* 2024), and teas (Jiang *et al.,* 2019), has proven antioxidant and antimicrobial properties. GA interferes with microbial physiology through membrane permeabilization, inhibition of nucleic acid synthesis, and suppression of biofilm formation (Liu *et al.,* 2017; Li *et al.,* 2017; Kang *et al.,* 2018a; Bai *et al.,* 2021). Despite its relatively low antimicrobial potency compared to tannic acid, GA's biological activity can be significantly enhanced through molecular modifications or nanoparticle delivery systems (Lee *et al.,* 2017; Shamsi *et al.,* 2018). Importantly, GA and its derivatives have been shown to exhibit synergistic antibacterial activity when used in combination with other antimicrobial agents (Gutiérrez-Fernández *et al.,* 2013), making it for integrative pest and pathogen control strategies.

Despite these findings, the potential synergy between insect-derived bio-foam and plant phenolics like TA and GA has not been explored. Therefore, the present study aims to explore the antimicrobial properties of *P. costalis* froth, its biochemical composition, and evaluate its synergistic potential when combined with TA and GA. The specific objectives of the study include: to record the presence of key excretory metabolites (urea, uric acid, creatinine) in the froth; to evaluate the insecticidal activity of the froth against three hemipteran pests; and also assess the microbicidal efficacy of the froth alone and in combination with

gallic acid and tannic acid. By integrating insect-derived defenses with plant polyphenols, this research seeks to develop a novel, nature-based strategy for crop protection and microbial control.

MATERIALS AND METHODS

Collection and maintenance of Spittlebug

The present study was conducted from December 2022 to March 2023 at two locations in Tamil Nadu: the St. Xavier's College campus, Palayamkottai (8.716211° N, 77.739315° E), and Puthukuruchi (8.608658° N, 77.765709° E) in Tirunelveli District. During the study period, biofoam secreted by nymphs of *P. costalis* was collected. Spittlebug foam nests were observed to vary in size depending on the host plant. On the college campus, spittlebug foam was mainly found on *Chloris barbata* Swartz (Poales: Poaceae) and *Ocimum tenuiflorum* Linn. (Lamiales: Lamiaceae) Other plant species also hosted spittlebugs during the nymphal stage, but those species were excluded from the study due to the presence of different spittlebug species. Spittlebug foam typically formed at two plant positions: the node and the leaf tip or stem. In some plants, up to three nymphs at various stages (1 to 3) were observed. The foam was collected using sterile 1.5 mL Eppendorf tubes and stored for further analysis.

Measurement of foam nest size

After removing the nymphs from the foam, the size of the foam nests was measured. Foam length ranged from 0.5 cm to 3.5 cm, while width was measured at three points: upper, center, and lower. If all three widths were identical, a single value was recorded. The average values of length and width were used to calculate the area using the formula:

Area (cm^2) = Length × Width

Spittlebug foam nest weight

Each foam nest was individually weighed. First, the empty Eppendorf tube weight (A) was recorded using a Mono Pan Balance (Dhona, India). Then, after adding the biofoam, the total weight (B) was measured. The net weight of the foam was determined by subtracting A from B.

Preservation and storage of foam

Preservation: Collected foam was mixed with 0.5–0.7 mL of DMSO4 in Eppendorf tubes and stirred. Samples were exposed to UV–C germicidal light for 5–10 minutes in a laminar airflow chamber. Sterilized foam was stored at -20°C. Storage feasibility analyses: Foam was stored at different temperatures: -20°C (deep freezer), 4°C (refrigerator), and 30–32°C (room temperature). Spectrometric optical density (OD) readings at 600 nm were recorded every 24 hours over eight days. Six replicates were maintained for each condition.

Quantitative estimation of metabolic

To analyze nitrogenous waste (urea, uric acid, creatinine), 1 g of foam was homogenized in 10 mL of 0.9% NaCl and centrifuged at 4000 rpm for 5 minutes. The supernatant was used for analyses of Urea (Kaplan,, 1969), Uric acid (Caraway, 1963), and Creatinine (Bonsnes and Taussky, 1945).

Insecticidal activity

Insects including *Aphis craccivora* C.L. Koch (Hemiptera: Aphididae) adults, *Dysdercus koenigii* Fabricius (Hemiptera: Pyrrhocoridae) second-instar nymphs, and *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) adults were collected from Kuthukkal (8.675820° N, 77.799712° E) Tirunelveli cotton fields and reared under laboratory conditions. A leaf-disc method was used for repellent bioassays at concentrations of 0.05%, 1%, 2%, 4%, and 8% foam. Each leaf disc (1 cm^2) was treated with 1 mL of foam solution, while a control disc was treated with DMSO4. Ten test insects were released in a Petri dish for each treatment. Insect distribution was recorded at intervals up to 30 minutes, and Access Proportion Index (API) was calculated.

Antimicrobial activity

Microbes including *Pseudomonas aeruginosa* Walter Migula (Pseudomonadales: Pseudomonadaceae), *Proteus vulgaris* Gustav Hauser (Enterobacterales: Enterobacteriaceae), *Klebsiella pneumonia* Carl riedlander (Enterobacterales: Enterobacteriaceae), *Xanthomonas malvacearum* Smith

(Xanthomonadales: Xanthomonadaceae), *and Xanthomonas citri* Hasse (Xanthomonadales: Xanthomonadaceae), were obtained from CPRC and maintained on nutrient agar slants.

Agar well diffusion method: The antibacterial activity of the spittlebug foam was tested using the agar well diffusion method. Sterile Petri dishes (9 cm in diameter, 1 cm in height) were filled with 20 mL of Mueller–Hinton agar (MHA) and allowed to solidify. Then, 100 µL of bacterial cultures *P. aeruginosa*, *P. vulgaris*, *K. pneumoniae*, *X. malvacearum*, and *X. citri* were evenly spread using a sterile L-rod. Using a sterile cork borer, 6 mm wells were punched in the agar. Each well was filled with 100 µL of test foam solution at different concentrations (10%, 20%, 40%, 80%, and 100%). Chloramphenicol (0.03%) served as the positive control, and DMSO4 was used as the negative control. Each treatment was replicated six times. After incubation for 24 hrs at 27°C in a BOD incubator (Kemi, Mudickal, India), the clear zones around each well (indicating bacterial inhibition) were measured. The percentage inhibition (PI) was calculated using the formula:

$PI = 100 \times (x - y) / (z - y)$

Where: x = inhibition area of test extract; y = inhibition area of negative control; z = inhibition area of positive control.

Statistical Analysis

Data from foam size, area, weight, and antimicrobial activity were analyzed using ANOVA at a 5% significance level. Variance homogeneity was checked, and non-normal data were log-arcsine transformed. ANOVA was also used to analyze different concentrations of biofoam and controls across microbial species.

RESULTS AND DISCUSSION

Poophilus costalis and froth formation

Poophilus costalis is widely distributed across Asia, from Iran to China and Korea, extending northward to the deserts of Kazakhstan, and found throughout most African countries (Mozaffarian and Wilson, 2015). The spittlebug's biofoam is commonly collected from grasses and other plants, with a clear preference for monocotyledonous grasses. Recently, this species has also been recorded on *Cannabis sativa* L. (Rosales: Cannabaceae), *Daucus carota* L. (Apiales: Apiaceae), and *Rumex hastatus* D. Don (Caryophyllales: Polygonaceae) (Mehraj *et al.*, 2023). Although various biological activities such as repellent, antibacterial, and antifungal effects have been observed in the froth of several spittlebug species, specific studies on *P. costalis* are limited (Del Campo *et al.*, 2011; Chang *et al.*, 2019b; Tonelli *et al.*, 2019, 2020; Sahayaraj *et al.*, 2021; Sahayaraj *et al.*, 2025).

Froth characteristics and storage

The froth is secreted by the nymphs of *P. costalis* from the anus and serves multiple purposes, including protection from desiccation and predators. The main component of the froth is xylem sap, consumed in large quantities by the insect to extract nutrients, with the excess excreted as froth (Wilson and Dorsey, 1957). The froth also plays a thermoregulatory role (Sahayaraj *et al.*, 2021). The study examined froth storage under various conditions (room temperature, refrigeration, deep freezing). Optical Density (OD) measurements revealed increased values up to 4 days, followed by a gradual decrease (Figure 1).

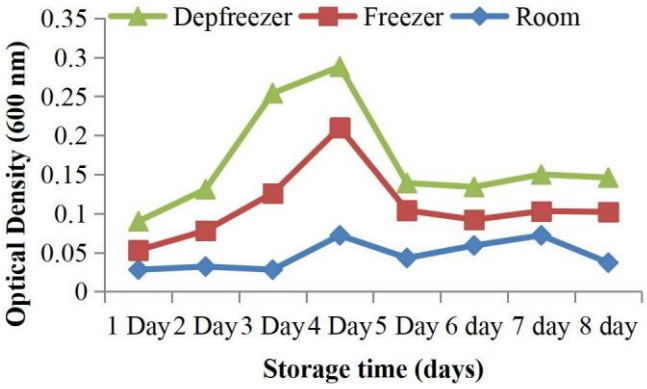

Figure 1. Optical Density (OD) of *Poophilus costalis* froth stored at room temperature, refrigerator and deep freezer (-20°C)

This is a first attempt; however, the results are considered important for future studies. The froth became less stable as the temperature increased. When the temperature went above 50°C, the surfactants in the froth began to break down and form solid particles, making the froth less stable

(Choung *et al.*, 2004). The froth is made up of liquid, air, and special molecules that help reduce surface tension and form foam (Marshall, 1973; Mello *et al.*, 1987). Tests showed that the froth contains proteins, carbohydrates, and lipids. Among these, proteins were found in most of the samples, while lipids were less common.

Excretory products in froth

The froth of *P. costalis* was analyzed for metabolic waste products. Past studies in insects like *Lucilia sericata* Meigen (Diptera: Calliphoridae) showed continuous production of uric acid and allantoin (Brown, 1938). In this study, urea, uric acid, and creatinine were detected for the first time in *P. costalis* froth. ANOVA results showed significant variation among metabolites (F = 8.9885, p = 0.002717) (Figure 2). Tukey's HSD test showed significant differences between urea and uric acid (p = 0.00506), and urea and creatinine (p = 0.00729) at 5% level, but not between uric acid and creatinine (p = 0.98179). These findings highlight the nitrogenous metabolic pathways in *P. costalis* froth. First time we recorded urea, uric acids and creatinine in the froth of *P. costalis*.

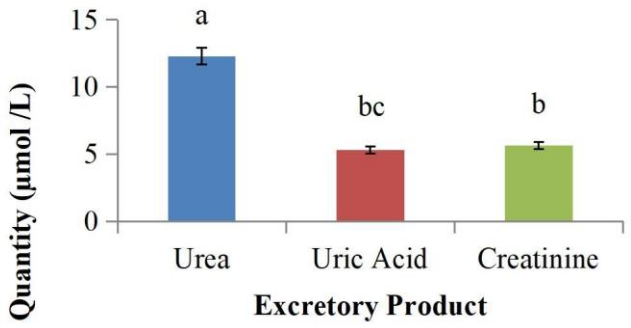

Figure 2. Excretory products (urea, uric acid and creatinine- µmol /L) recorded from the biofoam of *Poophilus costalis*

Insecticidal activity

Insecticidal activity includes either repellence or attractant. Tests were conducted on three cotton pests: *P. solenopsis*, *D. koenigii*, and *A. craccivora*. In a choice test, DMSO4 attracted *P. solenopsis* and *D. koenigii* but repelled *A. craccivora*. In contrast, *P. costalis* froth repelled *D. koenigii* and *A. craccivora*, but attracted *P. solenopsis* (except at 0.05%).

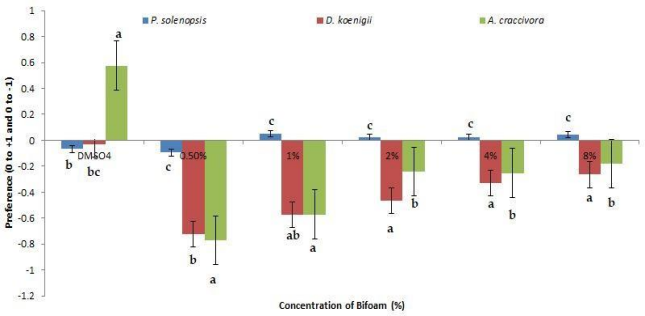

Figure 3. Attractant and repellent activity of *Poophilus costalis* biofoam at different concentrations (0.05, 1.0, 2.0, 4.0 and 8%) along with its solvent $DMSO_4$

Statistical analysis (Tukey's test) showed insignificant results for *P. solenopsis* (F = 0.21352; p = 0.928449) and *D. koenigii* (F = 1.44926; p = 0.24735). However, the results were significant for *A. craccivora* (F=24.74494; p=0.00001), especially among different concentrations (T1-T6) (Figure 3 and Table 1). These findings agree with Balzani *et al.* (2023), who found that the froth of *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) helps protect against predators. Other studies also show that spittlebug froth has many biological uses, such as acting as a repellent (Del Campo *et al.*, 2011). The froth not only keeps away predators but also repels other insects (Tonelli *et al.*, 2019). Therefore, it is important to understand what chemicals are present in the froth.

Microbicidal Activity

Phytopathogens

The froth, alone or combined with gallic or tannic acid, was tested against *X. malvacearum* and *X. citri* (Table 1 and Figure 4). The zone of inhibition was used to measure antimicrobial activity, which was dose-dependent. Combination treatments showed varied results: tannic acid-froth reduced *X. citri* more, while gallic acid-froth was more effective against *X. malvacearum*.

Previous reports revealed that froth contains chitinases like N-acetylglucosaminidase, chitobiosidase, and endochitinase, responsible for fungal growth suppression (Chang *et al.*, 2019b). Additionally, antimicrobial peptides (AMPs) from *Bacillus amyloliquefaciens* are known to produce

Table 1. Impact of spittlebug biofoam alone and in combination with gallic acid, tannic acid on the growth of two phytopathogens, *X. malvacearum* and *X. citri.*

Concentrations (%)	Froth		Gallic Acid + Froth		Tannic Acid+ Froth	
	X. malvacearum	***X. citri***	***X. malvacearum***	***X. citri***	***X. malvacearum***	***X. citri***
100	11.6±0.2^{b} (52.25%)	8.5±0.2^{b} (40.48%)	15.6±0.2^{b} (56.98%)	16.6±0.3^{b} (55.38%)	15.3±0.2^{b} (56.11%)	14.2±0.1^{b} (54.15%)
80	9.3±0.2^{c} (41.82%)	4.3±0.2^{c} (20.48%)	11.8±0.2^{c} (43.05%)	10.7±0.2^{c} (40.26%)	11.33±0.3^{c} (41.44%)	10.3±0.2^{c} (39.50%)
40	5.5±0.2^{d} (24.77%)	2.0±0.0^{d} (9.52%)	8.3±0.2^{d} (30.33%)	7.3±0.2^{d} (27.68%)	7.6±0.2^{d} (28.09%)	6.8±0.2^{d} (26.11%)
20	1.5±0.2^{e} (6.76%)	0.3±0.2^{e} (1.42%)	5.5±0.2^{e} (19.99%)	4.3±0.2^{e} (16.35%)	4.6±0.2^{e} (17.09%)	3.5±0.2^{e} (13.39%)
10	0.0±0.0^{f}	0.0±0.0^{f}	2.5±0.2^{f} (9.10%)	3.8±0.1^{f} (6.90%)	2.2±0.3^{f} (7.87%)	1.5±0.2^{f} (5.74%)
$DMSO_4$	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}
Chloramphenicol (0.031)	22.2±0.4^{a}	21.0±0.4^{a}	27.5±0.2^{a}	27.5±0.2^{a}	27.3±0.3^{a}	26.2±0.2^{a}

Significant differences in concentrations for the same microbe are indicated by the same lowercase letters

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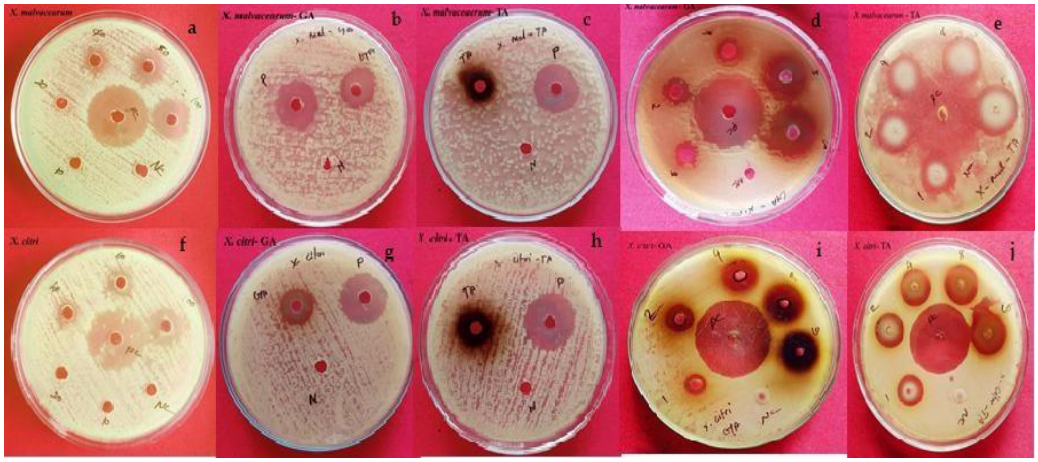

Figure 4. Impact of *Poophilus costalis* bioform at different concentrations (10, 20, 40, 80 and 100 %) (a, f), galic acid (b and g), tannic acid (c, and h), bioform + galic acid (d and i), and bioform + tannic acid (e, and j), and Chloramphenicol against two plant bacterial pathogens namely *Xanthomonas* m*alvacearum (a-e) and Xanthomonas* citri (f to j)

antibiotics such as iturin, bacilysin, bacillomycin, fengycin, surfactin, subtil (Sampathkumar *et al.*, 2023).

Long-chain fatty acids, known for antimicrobial activity, were also found in *P. costalis* froth (Sahayaraj *et al.*, 2021). However, both *X. malvacearum* and *X. citri* were not included in their review. First time, our research reveals, the fatty acids of spittle bugs showed anti-bacterial activity.The froth of *P. costalis* demonstrated strong antibacterial activity against Gram-positive human pathogens, especially *E. faecalis*, followed by *S. aureus* and *B. subtilis* (Table 2 and 3 and Figure 5). This activity was found to be dose-dependent, with higher concentrations yielding greater bacterial inhibition. When gallic acid was combined with the spittlebug froth, the antibacterial effect increased, particularly against *E. faecalis*. Similarly, the combination with tannic acid showed enhanced inhibition of *S. aureus*. Interestingly, chloramphenicol, used as a positive

control, unexpectedly promoted the growth of all three Gram-positive bacteria in this study.

Table 2. Impact of spittlebug biofoam on the growth of three G+ human pathogenic bacterium *B. subtilis* and *E. faecalis*

Concentration (%)	*B. subtilis*	*E. faecalis*
100	9.50±0.22[b] (44.55%)	7.66±0.21[b] (41.13%)
80	7.50±0.22[c] (35.17%)	5.83±0.16[c] (31.23%)
40	4.50±0.22[d] (21.06%)	9.83±0.16[d] (20.51%)
20	1.66±0.33[e] (7.82%)	1.83±0.16[e] (9.84%)
10	0.16±0.16[f] (0.80%)	0.66±0.21[f] (3.60%)
$DMSO_4$	0.00±0.00[f]	0.00±0.00[f]
Positive control Chloramphenicol (0.031)	21.33±0.21[a]	18.66±0.21[a]

Significant differences in concentrations for the same microbe are indicated by the same lowercase letters

This suggests possible bacterial resistance or interference by froth components. While previous studies have shown that tannic acid alone can inhibit *S. aureus* (Tintino *et al.,* 2016), our results indicated that its combination with froth did not have the same effect, warranting further investigation into their interaction. Additionally, fatty acids like palmitic and stearic acid classified as saturated fatty acids (SFA) have shown varying degrees of antibacterial activity against *P. aeruginosa*, and *S. aureus* (Huang *et al.,* 2014). Nanostructures of these acids were found to be effective against multidrug-resistant strains like *Staphylococcus epidermidis* Evans. (Bacillales: Staphylococcaceae) and vancomycin-resistant *E. faecalis* when encapsulated in liposomal carriers (Cheung Lam *et al.,* 2016). These formulation

Table 3. Impact of spittlebug biofoam + gallic acid, and biofoam tannic acid on the growth of three G+ human pathogenic bacterium *B. subtilis,* and *E. faecalis.*

Concentration (%)	*B. subtilis*	*E. faecalis*	*B. subtilis*	*E. faecalis*
	Gallic acid + Froth		Tannic acid + Froth	
100	14.83±0.30[b] (51.47%)	14.33±0.21[b] (48.60%)	14.00±0.25[b] (49.44%)	14.83±0.16[b] (52.36%)
80	12.33±0.21[c] (42.79%)	11.16±0.16[c] (37.87%)	11.50±0.22[c] (40.59%)	11.50±0.22[c] (40.59%)
40	8.66±0.21[d] (30.10%)	8.33±0.21[d] (28.25%)	8.16±0.16[d] (28.83%)	8.33±0.21[d] (29.41%)
20	6.16±0.16[e] (21.39%)	5.83±0.16[e] (19.78%)	6.16±0.16[e] (21.77%)	5.66±0.21[e] (20.01%)
10	3.50±0.22[f] (12.13%)	3.66±0.21[f] (12.43%)	3.16±0.16[f] (11.18%)	2.50±0.22[f] (8.82%)
$DMSO_4$	0.00±0.00[g]	0.00±0.00[g]	0.00±0.00[g]	0.00±0.00[g]
Chloroamphenical (0.031)	28.83±0.30[a]	29.50±0.22[a]	28.33±0.21[a]	29.33±0.21[a]

Significant differences in concentrations for the same microbe are indicated by the same lowercase letters

achieved minimum inhibitory concentrations (MICs) as low as 0.25–2 µg/mL. However, other studies reported no antibacterial activity of palmitic acid against either Gram-positive or Gram-negative bacteria (Sanabria-Rios *et al.,* 2015; Carballeira *et al.,* 2017; Sanabria-Ríos *et al.,* 2020). These contradictory findings might be due to differences in bacterial strains or experimental conditions. Overall, the biofoam of *P. costalis*, especially when combined with plant-based compounds like gallic or tannic acid, offers promising antimicrobial potential and deserves further exploration.

The froth of *P. costalis* exhibited notable antibacterial activity against Gram-negative human pathogens. Among the tested strains, *E. coli* showed the highest growth suppression, followed by *P. aeruginosa*, *K. pneumoniae*, and *P. vulgaris* (Table 4 and Figure 6). However, Nitbani *et al.* (2016) observed that 15% v/v lauric acid exhibited stronger antibacterial activity against *S. aureus*, *Bacillus cereus* Frankland (Bacillales: Bacillaceae), *Salmonella typhimurium* (Enterobacterales: Enterobacteriaceae), and *E. coli* than 0.5% v/v ciprofloxacin. The observed resistance of certain pathogens may also be linked to tannin tolerance. As tannins are common in the human diet, they can influence the composition of gut microbiota and affect bacterial growth (Chung *et al.*, 1998). These findings indicate the need for further studies to understand the mechanisms behind the antimicrobial action of spittlebug froth and its interaction with other bioactive compounds. The froth of *Poophilus costalis* exhibits multifunctional properties, including thermoregulation, repellence, and strong antimicrobial activity. It contains proteins, fatty acids, and metabolic waste like urea, uric acid, and creatinine. The froth demonstrated dose-dependent antibacterial effects, especially when combined with gallic or tannic acid. Significant inhibition was observed against both G^+ and G^- pathogens. These findings highlight the biofoam's potential in agricultural and biomedical applications, deserving further investigation.

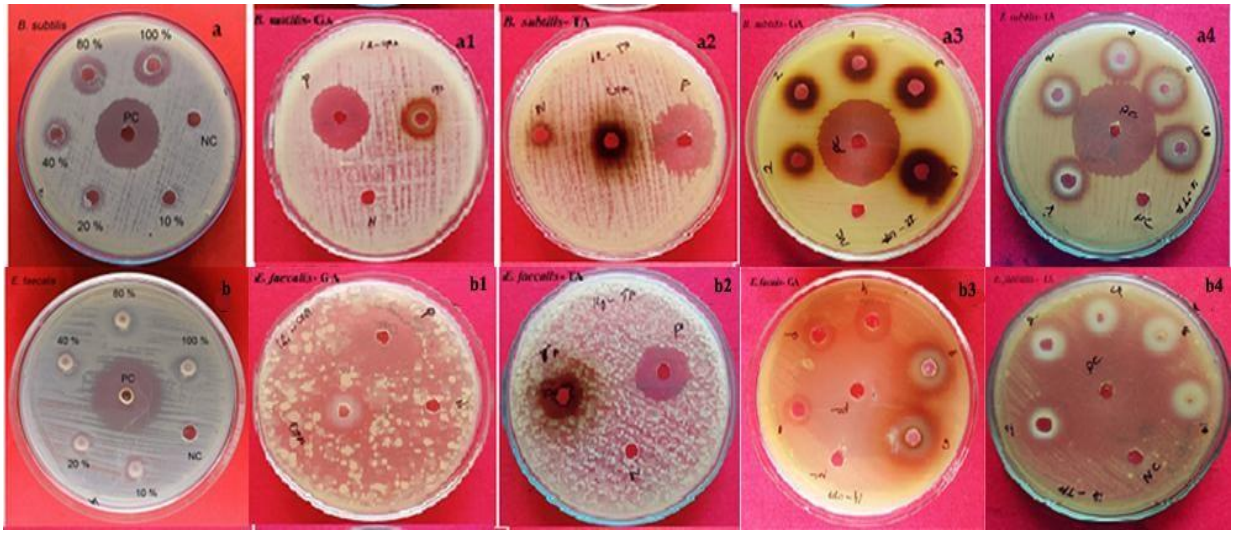

Figure 5**.** Impact of *Poophilus costalis* bioform at different concentrations (10, 20, 40, 80 and 100 %) (a and b), galic acid (a1 and b1), tannic acid (a2 and b2), bioform + galic acid (a3 and b3), and bioform + tannic acid (a4 to c4), and chloramphenicol against three human pathogenic G+ bacterium namely *Bacillus subtilis* (a1-a4) and *Enterococcus faecalis* (b1 to b4).

Table 4. Impact of spittlebug biofoam + gallic acid, and biofoam + tannic acid on the growth of three G- human pathogenic bacterium *E. coli*, *P. vulgaris*, *P. aeruginosa*, *K. pneumonia* and *E. aerogenes*

Concentration (%)	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*K. pneumonia*	*E. aerogenes*	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*K. pneumonia*	*E. aerogenes*
	Gallic acid + Froth					Tannic Acid + Froth				
100	15.5±0.2^{b} (53.53)	11.3±0.2^{b} (46.25%)	13.3±0.2^{b} (45.47%)	13.5±0.2^{b} (61.01)	13.6±0.2^{b} (51.25%)	13.8±0.3^{b} (48.56%)	16.6±0.2^{b} (52.81%)	14.6±0.21^{b} (57.53%)	15.6±0.2^{b} (56.96%)	13.6±0.2^{b} (50.00%)
80	12.1±0.1^{c} (41.98%)	9.1±0.1^{c} (37.41%)	10.5±0.2^{c} (35.79%)	10.5±0.2^{c} (47.46)	10.6±0.2^{c} (40.00%)	11.1±0.1^{c} (39.20%)	12.3±0.2^{c} (41.59%)	11.3±0.2^{c} (44.46%)	11.6±0.2^{c} (42.43%)	10.5±0.2^{c} (38.46%)
40	8.6±0.21^{d} (29.86%)	6.5±0.2^{d} (26.52%)	7.2±0.1^{d} (24.43%)	6.8±0.2^{d} (30.85%)	7.3±0.2^{d} (27.49%)	8.5±0.2^{d} (29.84%)	7.0±0.2^{d} (30.34%)	7.7±0.2^{d} (30.07%)	9.3±0.2^{d} (33.95%)	7.5±0.2^{d} (27.50%)
20	5.6±0.2^{e} (19.57%)	3.3±0.21^{e} (13.61%)	4.2±0.1^{e} (14.24%)	4.3±0.2^{e} (19.54%)	4.3±0.2^{e} (16.26%)	6.3±0.2^{e} (22.20%)	5.3±0.2^{e} (17.98%)	4.6±0.2^{e} (18.30%)	7.0±0.0^{e} (25.46%)	4.7±0.2^{e} (17.09%)
10	3.3±0.2^{f} (11.52%)	7.50±0.22^{f} (6.11%)	7.5±0.2^{f} (5.09%)	7.6±0.2^{f} (7.51%)	8.3±0.2^{F} (8.76%)	4.3±0.2^{f} (15.22%)	2.5±0.2^{f} (8.40%)	1.8±0.3^{f} (7.17%)	4.8±0.1^{f} (17.59%)	2.5±0.2^{f} (9.17%)
DMSO4	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{g}
Chloramphenicol (0.031)	29.0±0.3a	24.5±0.2^{a}	29.3±0.3^{a}	22.1±0.3^{a}	26.6±0.2^{a}	28.5±0.22 a	29.6±0.21 a	25.5±0.2^{a}	27.5±0.2^{a}	27.3±0.3^{a}

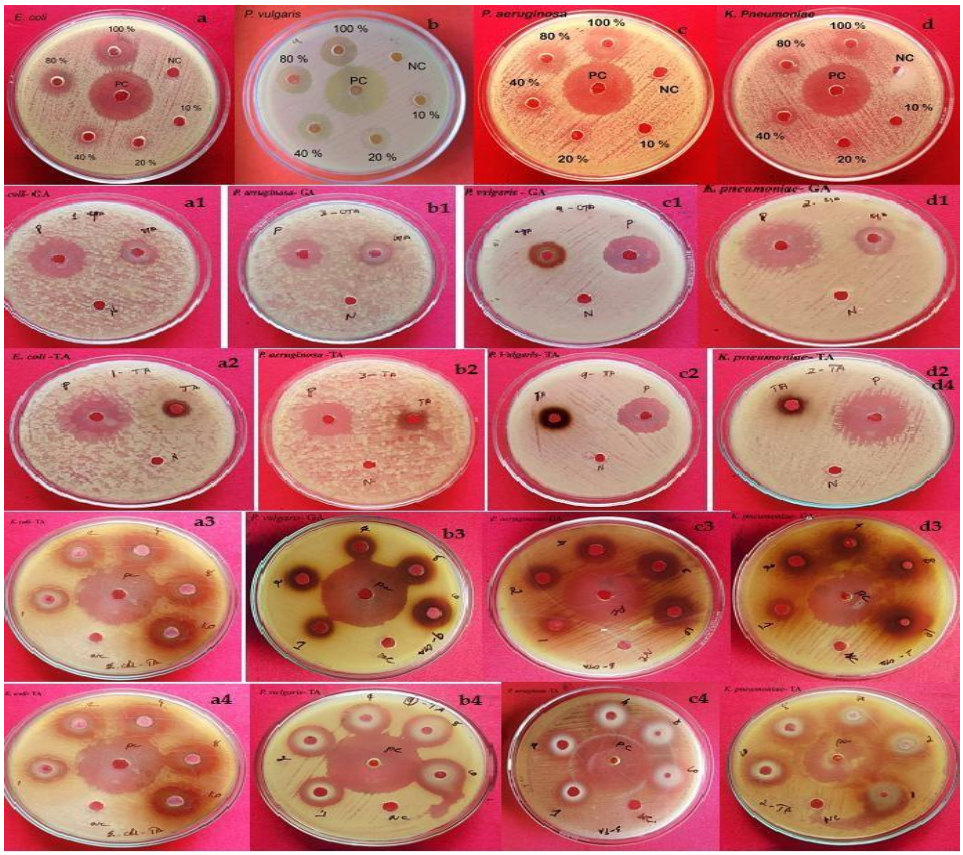

Figure 6. Impact of *Poophilus costalis* bioform at different concentrations (10, 20, 40, 80 and 100 %) (a to d), of galic acid (a1-d1), tannic acid (a2 to d4), bioform + galic acid (a3 to d3), and bioform + tannic acid (a4 to d4), and chloramphenicol against three human pathogenic G- bacterium namely *Escherichia coli (a1-a4)*, *Proteus vulgaris* (b, c1, c2, b3, b4), *Pseudomonas aeruginosa* (c, b1, b2, c3, c4) and *Klebsiella pneumoniae* (d to d4).

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