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Application of *Piper betle* Leaf Extract as a Bioactive Additive in Eco-Friendly Antifouling Coatings

Nguyen Duc Anh, Cao Nhat Linh *, Le Thi My Hiep and Dong Van Kien

Coastal Branch of Joint Vietnam-Russia Tropical Science and Technology Research Center, 30 Nguyen Thien Thuat, Nha Trang 57127, Vietnam; nda.ttndvn@gmail.com (N.D.A.); myhiepru@gmail.com (L.T.M.H.); vankien29@gmail.com (D.V.K.)

* Correspondence: cnlinh0812@vrtc.org.vn; Tel.: +84-862-45-26-09

Abstract

The present study aimed to evaluate the antifouling efficacy of *Piper betle* leaf extracts as a bioactive additive for eco-friendly antifouling coatings. The composition of *P. betle* extract was determined and analyzed. Phytochemical analysis revealed that the ethanol extract of *P. betle* contained phenolics, tannins, proteins, carbohydrates, and flavonoids, with total phenolic content reaching 260.3 mg GAE/g dry weight and flavonoid content reaching 52.56 mg QE/g dry weight. The antibacterial test results showed that the ethanol extract of *P. betle* exhibited maximum antibacterial efficacy against *E. coli*, *B. subtilis*, *S. aureus*, and marine bacteria, with inhibition zone diameters of 28.7 ± 0.5, 27.0 ± 1.6, 22.1 ± 0.6, and 35.1 ± 0.5 mm, respectively. Based on the laboratory test results, the ethanol extract of *P. betle* was chosen to be added to coatings as an antifouling additive. The content of the extract was 0.5, 1.0, and 1.5 wt.%. A field test conducted in tropical seawater (at Nha Trang Bay) demonstrated that incorporating 1 wt.% of *P. betle* extract into an acrylic copolymer-based coating significantly enhanced its antifouling performance. After nine months of immersion in seawater, this sample maintained an antifouling efficiency of 74%. These findings highlight the potential of *P. betle* extract as a sustainable alternative to conventional antifouling agents in marine coatings.

Keywords: *Piper betle* extract; antibacterial activity; antifouling coatings; marine biofouling

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1. Introduction

Biofouling refers to the undesirable accumulation of biological organisms—microorganisms, barnacles, mussels, algae, and other marine invertebrates—on the surfaces of materials continuously immersed in seawater. This phenomenon has several negative consequences, such as increased hydrodynamic drag and structural loads on vessels and offshore structures, reduced navigational efficiency and speed, elevated fuel consumption, material degradation through corrosion, and overall reduction in service life [1].

In the context of expanding maritime operations, developing environmentally benign antifouling coatings is imperative for the protection of marine ecosystems as well as for ensuring the sustainability of aquaculture and marine engineering industries [2,3]. Among the various antifouling strategies, applying antifouling coatings remains the most prevalent approach due to their high efficacy, ease of implementation, and relatively low cost. Copper-based compounds, such as copper flakes and copper oxides, are among the most commonly employed active agents in these coatings [4,5]. In addition, synthetic biocides, including

Irgarol 1051, DCOIT, dichlofluanid, and chlorothalonil, are widely incorporated as antifouling agents [6]. Nevertheless, these synthetic and metal-based biocides exhibit varying degrees of ecotoxicity, raising concerns regarding their environmental persistence and impact. As a result, regulatory authorities in several countries have imposed restrictions on the use of copper compounds and synthetic toxicants in antifouling formulations [7]. In response to increasingly stringent environmental standards, developing eco-friendly antifouling coatings—non-toxic or based on naturally derived bioactive agents—has attracted growing scientific attention [8–10].

One promising approach involves the use of plant-derived compounds as natural antifouling additives. Extracts may be derived from various plant parts, including roots, leaves, fruits, bark, and seeds. Plant extracts are rich in polyphenolic compounds—more than 8000 polyphenol types have been identified—classified as flavonoids, phenolic acids, polyphenolic amides, and others [11,12]. These compounds possess high antimicrobial and antifouling properties and have been extensively applied in pharmacy, medicine, and in the development of bio-based antifouling coatings. Notable examples include extracts from *Zingiber officinale* R. (ginger root) [13], *Nerium oleander* L. (oleander) [14], capsaicin from chili peppers [15–17], and extracts from the leaves of *Sonneratia lanceolata* B. [18].

Piper betle Linn. (*P. betle*) is a plant species widely distributed throughout South and Southeast Asia. Its leaves contain high concentrations of bioactive compounds with potent antimicrobial properties and are traditionally utilized in food preservation and medicinal applications [19]. Phytochemical investigations have shown that *P. betle* extracts contain several bioactive compounds, including phenolics, alkaloids, saponins, tannins, and flavonoids [20,21]. Compared to other plant extracts, the *P. betle* extract showed potent antibacterial activity against Gram-negative and Gram-positive bacterial strains [22–25]. Studies have also shown that phenolic compounds such as hydroxychavicol and allylpyrocatechol, identified in the *P. betle* extract, have been reported to exhibit anti-plaque activity by compromising the integrity of the cell wall of *Streptococcus sanguinis*, a primary bacterium involved in dental plaque formation [26]. In addition, the *P. betle* extract has high antibacterial activity and anti-biofilm potential against *S. pseudintermedius* and *E. coli* [27,28]. This makes the *P. betle* extract a promising candidate for incorporation into environmentally friendly antifouling coatings. However, there have been no reports on using *P. betle* extract as an additive in antifouling coatings.

This study investigates the use of *P. betle* extract as a natural antifouling additive in the formulation of non-toxic, bio-based coatings intended to replace conventional biocide-based systems. The *P. betle* extract is analyzed to determine the content of certain organic functional groups and then is tested to evaluate their antibacterial activity. The extract is incorporated into an acrylic-based coating and applied onto steel panels. The coated samples are thereafter subjected to an antifouling efficacy investigation. In addition, the morphology and surface properties of the coating are also examined.

2. Materials and Methods

2.1. Preparation of P. betle Extracts

Piper betle Linn. leaves were collected from Nha Trang City, Khanh Hoa Province, Vietnam (12.2385° N, 109.159° E) on 16 September 2024 and were identified by the Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh city. The voucher specimens 21,002 have been deposited in the Coastal Branch of the Joint Vietnam–Russia Tropical Science and Technology Research Center. The leaves were thoroughly washed and dried at 45 °C for 72 h, then ground into fine powder using a mechanical grinder (PG2500, Spring Green Evolution, Bangkok, Thailand). The powders (500 g) were macerated in various solvents (methanol, ethanol, and ethyl acetate)

at 1:10 (*w*/*v*) for 24 h at 25 °C. This procedure was repeated three times to maximize the extraction. The mixture was filtered using filter paper, and then the combined filtrates were concentrated using a rotary vacuum evaporator (R215, Buchi, Switzerland) at 40 °C under reduced pressure (175 mbar for ethanol, 337 mbar for methanol, and 240 mbar for ethyl acetate). The concentrated extracts were vacuum-dried at 45 °C using a vacuum oven (SH VDO-125NG, SH Scientific, Sejong-si, Republic of Korea) until the solvent was removed. The crude extracts were stored at 4 °C for subsequent experimental procedures. In the current study, the extraction yielded 70.5 g of the crude extract of *P. betle.*

2.2. Extracts Characterization

2.2.1. Fourier Transform Infrared (FTIR) Analysis

The extracts were characterized for chemical structure by an FTIR spectrometer (Agilent Cary 630, Agilent Technologies, Malaysia) in the range of 4000–630 cm^{-1}.

2.2.2. Determination of the Presence of *P. betle* Extract Compounds by Phytochemical Screening

To determine the presence of phytochemical constituents such as phenolics, tannins, proteins, carbohydrates, and flavonoids in crude extract, the test was done according to [29]. The concentrated extracts were dissolved in ethanol to obtain a 100 mg/mL concentration.

Phenolic compound test: 2 mL of the filtered extract was added to a 1% $FeCl_3$ solution to detect phenolic compounds. The appearance of a dark blue or greenish-black coloration indicated the presence of phenolic compounds.

Tannins test: 2 mL of the filtered extract was mixed with a 1% lead acetate [$Pb(C_2H_3O_2)_2$] to test for tannins. The formation of a gelatinous precipitate was taken as evidence of tannin presence.

Protein test: Proteins were detected using the Biuret test. 2 mL of the filtered extract was treated with a drop of 2% $CuSO_4$ solution and 1 mL of 95% ethanol. A small amount of KOH was then added. The formation of a pink-colored precipitate indicated the presence of proteins.

Carbohydrate test: Carbohydrates were detected by gradually treating 2 mL of the filtered extract with a few drops of diluted sulfuric acid (H_2SO_4). The appearance of a purple precipitate confirmed the presence of carbohydrate compounds.

Flavonoids test: 2 mL of the filtered extract was treated with a small amount of metallic magnesium to assess the presence of flavonoids, followed by a few drops of concentrated hydrochloric acid (HCl). The development of a red coloration suggested the presence of flavonoid compounds.

2.2.3. Determination of the Total Phenolic Content (TPC) and the Total Flavonoid Content (TFC)

The total phenolic content (TPC) was determined following the method described in [30]. According to that, 2.5 mL of 10% (*v*/*v*) Folin–Ciocalteu reagent in distilled water was mixed with 0.5 mL of the extract (diluted with ethanol to obtain a concentration of 5 µg/mL). After 6 min, 2 mL of 7.5% (*w*/*v*) Na_2CO_3 solution was added to the mixture, which was then left in the dark for one hour at room temperature. The mixture absorbance was measured at 760 nm using a UV-Vis spectrophotometer (DR3900, Hach, Loveland, CO, USA). Gallic acid was used as a quantification standard, and the TPC results were expressed in milligram gallic acid equivalents (GAE) per gram of dry weight. The total flavonoid content (TFC) was determined using the $AlCl_3$ colorimetric method. Dimethyl sulfoxide (DMSO) was used as the solvent to dilute and prepare a concentration series of the crude extracts. Specifically, 0.5 mL of *P. betle* extract (at a concentration of 100 µg/mL) was mixed with 1.5 mL of ethanol and allowed to stand for 5 min. Subsequently, 0.1 mL of 10% $AlCl_3$ solution was added, and the mixture was shaken gently. After a 6-min reaction, 0.1 mL of 1 M CH_3COOK and 2.8 mL of distilled water were added. The mixture was

thoroughly shaken and incubated at room temperature. After 30 min, the absorbance was measured at 415 nm using a UV-Vis spectrophotometer. Quercetin was used as a positive standard. The total flavonoid content in the *P. betle* extract was calculated in milligrams of quercetin (QE) per gram of dry weight [31].

2.2.4. Antibacterial Activity Test

The antibacterial activity of *P. betle* extract was evaluated against three bacterial strains: *Escherichia coli* ATCC 15034, *Bacillus subtilis* BKM B501, and *Staphylococcus aureus* ATCC 21027. Bacterial strains are supported at –85 °C in cryotubes in the Collection of Heterotrophic Bacteria, Coastal Branch of the Joint Vietnam–Russia Tropical Science and Technology Research Center. In addition, the extracts were also assessed against marine bacteria. Seawater samples containing native marine microorganisms were collected from the Dam Bay Marine Research and Testing Station (12.197° N, 109.292° E) on 12 December 2024. Marine bacterial cultures were obtained by inoculating 0.1 mL of seawater onto Marine Broth agar plates. The extract samples (at a concentration of 300 μg/mL) were applied to wells (50 μL per well) created in the agar. Penicillin G (at a concentration of 6 μg/mL) was used as a positive control. The agar plates were incubated at 30 °C for 18 h. Five test runs on each sample were carried out. Antibacterial activity was assessed by observing the presence or absence of inhibition zones and measuring the diameter of the inhibition zones (in mm), which reflects the extent of microbial growth suppression.

2.3. Preparation and Characterization of Coatings

2.3.1. Paint Preparation

Paints were prepared by mixing the ingredients using a high-speed disperser (Alligator Machine, AAU Technology Trading Co., Ltd., Ho Chi Minh City, Vietnam). The formulation included 37.5 g of acrylic copolymer (butyl methacrylate, methacrylic acid, methyl methacrylate), 50 g of rosin, 7.5 g of oleic acid, 25 g of $CaCO_3$, 50 g of ZnO, 5 g of bentonite, and 250 mL of solvent (xylene). The ethanol extract of *P. betle* was added to the matrix at concentrations of 0.5, 1.0, and 1.5 wt.%.

2.3.2. Surface Morphology Analysis

The surface micromorphology of the coatings was examined using scanning electron microscopy (SEM), SNE-ALPHA (SEC Co., Ltd., Suwon, Republic of Korea).

2.3.3. Surface Wettability Analysis

The surface wettability of coatings was determined by advancing contact angle measurement using an optical contact angle measuring and contour analysis system, OCA 15EC (DataPhysics Instruments GmbH, Filderstadt, Germany). For each composition, three samples were tested.

2.3.4. Determination of the Self-Polishing Rate (SPR) of Coatings

The study of the self-polishing rate of the coatings was conducted under laboratory conditions over a 10-day immersion period in natural seawater, following the methodology described in [32]. Coating samples were prepared in square specimens (3 × 3 cm). After drying for 48 h, the initial mass of each specimen was measured using an analytical balance with a precision of 0.0001 g. The samples were then immersed in 150 mL of natural seawater in sealed containers and placed on an orbital shaker (Grant-bio PSU-20i, Grant Instruments Ltd., Royston, UK) with an operating speed of 120 rpm. For each composition, three replicate samples were tested. After 10 days of immersion, the samples were allowed to air-dry at room temperature for 48 h, and then the weight of the specimens

was recorded to assess their self-polishing rate. The self-polishing rate was calculated using the following equation:

$$SPR = \frac{W_0 - W_{t,dry}}{S.t} \quad (1)$$

where W_0—initial sample mass before immersion; $W_{t,dry}$—sample mass after drying post-immersion; S—surface area of the sample (cm^2); t—time of immersion (days).

2.3.5. Field Test of Coatings

To evaluate the antifouling performance of antifouling coatings, a real sea hanging test was conducted at the marine experimental station located at Dam Bay Marine Research and Testing Station (Nha Trang Bay, 12.197° N; 109.292° E). The average seawater parameters were as follows: temperature 29.24 °C, pH 8.27, dissolved oxygen 6.47 mg/L, and salinity 33.4‰. The coating samples were applied to Ct3 carbon steel panels with dimensions of 150 × 100 mm and exposed under full immersion conditions at a depth ranging from 0.5 to 1.5 m below the seawater surface. A coating sample without an antifouling agent was used as the negative control, while a coating containing 20 wt.% Cu_2O served as the positive control.

To perform preliminary observations of fouling, the experimental samples were taken out for 5–10 min, and their surfaces were photographed at high magnification. Subsequently, the photos of the samples were examined on a PC at maximum digital magnification. The physical performance of the coating surface was evaluated, and the area of the fouling surface of the samples was calculated using ImageJ 1.48v software (National Institutes of Health, Bethesda, MD, USA). The antifouling efficiency (AE) was assessed taking into account the species of organisms and fouling area according to the formula [33]

$$AE = 100 - (0.2 \times S_1 + 0.5 \times S_2 + 15 \times S_3) \quad (2)$$

where AE—antifouling efficiency (%), S_1—percent of area covered with slime; S_2—percent occupied by non-encrusting organisms; S_3—percent occupied by fouling organisms with hard shells larger than 5 mm. The higher the AE, the better the antifouling properties of the coating.

Statistical analysis. All results are presented as means ± standard deviation (SD). Statistically significant differences ($p < 0.05$) were determined using one-way ANOVA and Tukey's test.

3. Results and Discussion

To evaluate the composition of organic functional groups in the extracts, Fourier-transform infrared (FTIR) spectroscopy was performed. The results are presented in Figure 1. The spectra exhibit relatively consistent peak positions across the samples, indicating a similar composition of functional groups. Notable absorption bands were observed at the following wavenumbers: a broad peak in the range of 3600–3300 cm^{-1} corresponding to O–H stretching vibrations in carboxylic or hydroxyl groups; a peak at 2928 cm^{-1} attributed to the stretching vibration of C–H bonds in the methyl group on the aromatic ring; The presence of an aromatic ring is further confirmed by the presence of peaks at 1600–1400 cm^{-1}. The presence of phenolic hydroxyl groups is confirmed by absorption bands at 1263–1200 cm^{-1} [34].

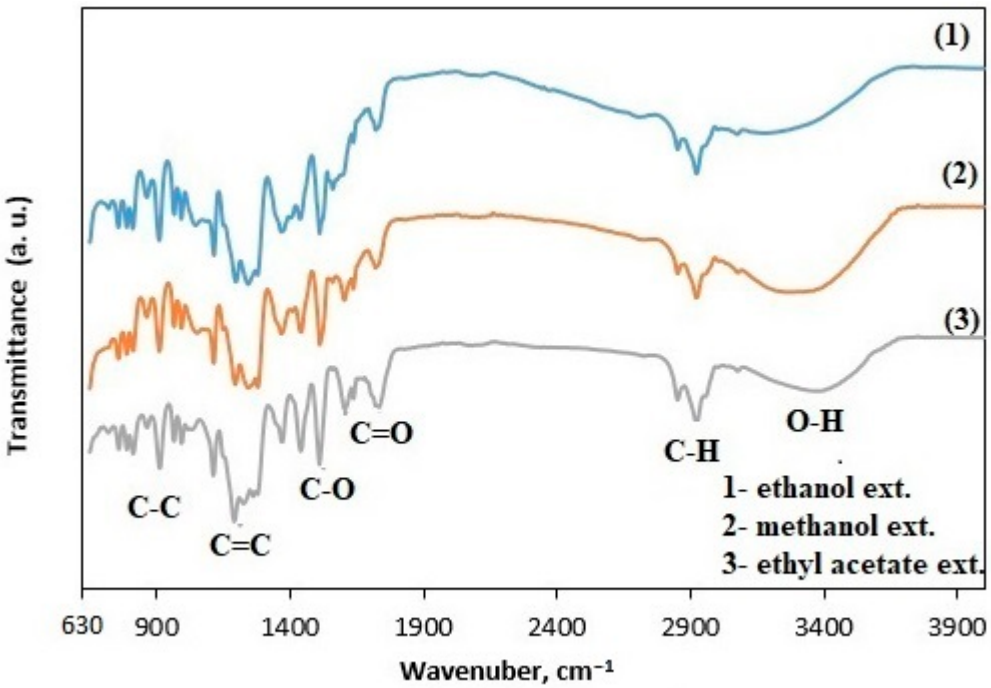


Figure 1. FTIR spectra of *P. betle* extracts obtained using different solvents.

As the FTIR spectral analysis revealed similar results across the extracts obtained using different solvents, the ethanol extract of *P. betle* was selected to analyze specific compound groups. The results of the phytochemical screening are shown in Table 1. Based on the screening, phenolics, tannins, proteins, carbohydrates, and flavonoids were detected. Compared with previous studies, Kaveti et al. [35] also reported the presence of several classes of compounds in *P. betle* extracts, including alkaloids, tannins, glycosides, and saponins. Meanwhile, *P. betle* collected in Mauritius contained phenols, flavonoids, and tannins [36]. Samples collected in India contained steroids, tannins, proteins, amino acids, flavonoids, terpenoids, saponins, and carbohydrates [37].

Table 1. Preliminary phytochemical screening of *P. betle* extract obtained using ethanol.

Phytochemical Group	Presence (+/−) [1]
Phenolics	+
Tannins	+
Proteins	+
Carbohydrates	+
Flavonoids	+

[1] *Note*: (+) detected; (−) not detected.

Phenolic and flavonoid compounds present in plant extracts have been widely demonstrated to exhibit potent antibacterial activity [14,38]. Therefore, analyses of total phenolic content (TPC) and total flavonoid content (TFC) were conducted for the ethanol extract of *P. betle* (Table 2). The results showed that the ethanol extract of *P. betle* contains a high concentration of phenolics (260.3 ± 5.22 mg GAE/g dry weight) and flavonoids (52.56 ± 1.26 mg QE/g dry weight). Similar results were also reported in the studies of Lam Thi Truc Nguyen et al. [39] on the use of different solvents (methanol, ethanol, ethyl acetate, and hexane) on *P. betle*, implying that ethanolic extracts had the maximum phenolic and flavonoid content (249.96 ± 6.42 mg GAE/g and 27.82 ± 1.25 RE/g). On the other hand, the *P. betle* extract collected in Mauritius had a lower TPC (129.43 ± 0.50 mg GAE/g), which may reflect differences in ecological conditions [36]. The chemical composition of *P. betle* extracts has been previously investigated in the literature. Several phenolic

constituents have been identified in *P. betle* extracts, including allyl pyrocatechol, allylcatechol, methyl eugenol, estragole (methyl chavicol), chavibetol, chavibetol acetate, safrole, 4-allyl-2-methoxy-phenol acetate, and 3-allyl-6-methoxyphenol [40,41].

Table 2. Total phenolic and flavonoid contents in the ethanol extract of *P. betle.* Data are presented as mean ± SD (n = 3).

Component	Results
Total phenolic content, mg GAE/g dry weight	260.3 ± 5.22
Total flavonoid content, mg QE/g dry weight	52.56 ± 1.26

The results of the antibacterial activity of *P. betle* extracts obtained using different solvents are presented in Figure 2 and Table 3. The results indicate that the ethanol extract showed high antibacterial activity against all tested bacterial strains. The inhibition zone diameters for *E. coli*, *B. subtilis*, *S. aureus*, and marine bacteria were 28.7 ± 0.5 mm, 27.0 ± 1.6 mm, 22.1 ± 0.6 mm, and 35.1 ± 0.5 mm, respectively. In contrast, the methanol extract demonstrated vigorous antibacterial activity specifically against marine bacteria, with an inhibition zone diameter of 33.0 ± 0.5 mm. The extract obtained using ethyl acetate exhibited the lowest antibacterial activity among all tested extracts against the evaluated bacterial strains. These results are consistent with previous studies. In [35], the authors reported that the ethanol extract of *P. betle* demonstrated the highest antibacterial efficacy. In addition, ethanol extracts at 50–100 µg/mL concentrations showed the largest inhibition zones against *E. coli* and *P. aeruginosa*. Compared to other plants, *P. betle* showed an outstanding antibacterial activity against the Gram-positive methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*, with the inhibition zones recorded at 17–33 mm [25]. According to the literature, the abundance of active phenolics and flavonoids may confer strong inhibitory effects of the extract against microorganisms [36,42].

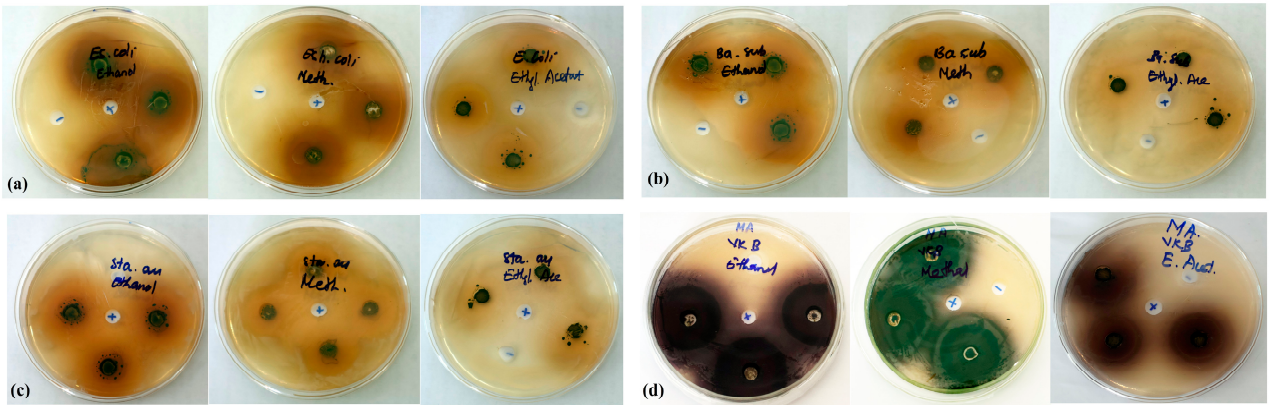


Figure 2. Inhibition zone of *P. betle* extracts against *E. coli* (**a**), *B. subtilis* (**b**), *S. aureus* (**c**), and marine bacteria (**d**). Symbols '+' and '–' indicate positive and negative controls, respectively.

Table 3. Antibacterial activity of *P. betle* extracts against *E. coli*, *B. subtilis*, *S. aureus*, and marine bacteria. Data are presented as mean ± SD (n = 5).

Extracts	Diameter of Inhibition Zone (mm)			
	E. coli	*B. subtilis*	*S. aureus*	*Marine bacteria*
Positive control	15.0 ± 0.5	11.4 ± 0.2	40.1 ± 0.6	42.3 ± 0.4
Ethanol extract	28.7 ± 0.5	27.0 ± 1.6	22.1 ± 0.6	35.1 ± 0.5
Methanol extract	23.3 ± 0.4	20.0 ± 0.2	18.1 ± 1.0	33.0 ± 0.5
Ethyl acetate extract	15.7 ± 1.8	12.3 ± 0.7	9.1 ± 1.0	20.0 ± 1.5

Based on the results of antibacterial activity, the ethanol extract of *P. betle* was selected for use as an antifouling agent in marine antifouling coatings. The extract was added to the acrylic-based coating matrix at concentrations of 0.5, 1.0, and 1.5 wt.%. The results of contact angle measurement of coatings are shown in Figure 3 and Table 4. It is seen that, as the extract's concentration increased, the hydrophobicity of the coating increased. At the highest concentration (1.5 wt.%), the contact angle increased to 73.2°, indicating a substantial enhancement compared to the control sample. This may be related to phenolic compounds in *P. betle* extract. A study by Md Sadek Ali et al. [43] shows that incorporating phenolic compounds from *P. betle* onto chitosan-based films can improve their hydrophobicity.

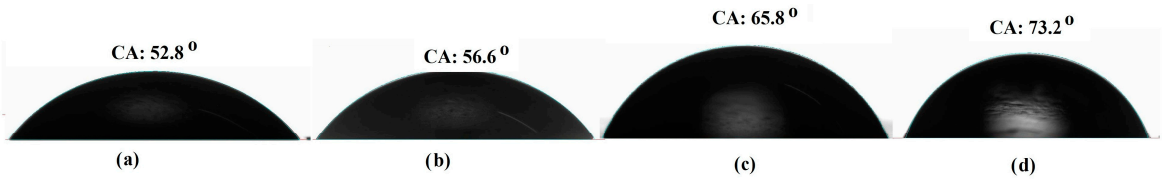


Figure 3. Contact angle of acrylic-based coatings: (**a**)—control coating without extract, (**b**)—coating with 0.5 wt.%, (**c**)—coating with 1 wt.%, and (**d**)—coating with 1.5 wt.% *P. betle* extract.

Table 4. Value of contact angle and self-polishing rates of antifouling coatings containing different *P. betle* ethanol extract concentrations. Data are presented as mean ± SD (n = 3).

Extract Concentration (wt.%)	Contact Angle (°)	Self-Polishing Rate (mg/cm^2·day)
0	52.8 ± 1.95	0.470 ± 0.012
0.5	56.6 ± 1.57	0.351 ± 0.011
1.0	65.8 ± 1.03	0.393 ± 0.015
1.5	73.2 ± 1.49	0.560 ± 0.018

The results of the self-polishing rate (Table 4) show that the self-polishing rate of the coating decreased with the addition of the extract at concentrations of 0.5 and 1.0 wt.%, but increased when the extract concentration was raised to 1.5 wt.%. These findings suggest that incorporating a small amount of *P. betle* extract enhances the coating's stability, potentially due to interactions between the organic functional groups in the extract and the polymer matrix, which may strengthen internal bonding and reduce the polymer degradation rate. However, at a higher extract concentration (1.5 wt.%), the excessive presence of organic compounds may disrupt the structural integrity of the polymer network, leading to a higher self-polishing rate.

The SEM images of the coating surfaces are presented in Figure 4. The surface of the control sample (without additive) appears relatively smooth but still exhibits some irregular defects and heterogeneities (Figure 4a). Compared to the control, the coatings containing *P. betle* extract showed a more uniform surface morphology. The extract was relatively well dispersed within the polymer matrix; however, it did not dissolve but instead formed microdroplets with diameters smaller than one micrometer (Figure 4b–d). These microdroplets were gradually released when immersed in seawater, acting as antifouling agents on the coating surface. At an extract concentration of 1.5 wt.%, the surface exhibits a noticeable increase in the number and size of voids or dispersed domains (Figure 4d). This phenomenon indicates possible phase incompatibility or oversaturation of the extract, which may lead to disruption of the polymer matrix. The increased porosity and heterogeneity will likely result in a higher self-polishing rate and reduced structural stability, consistent with previous experimental findings.

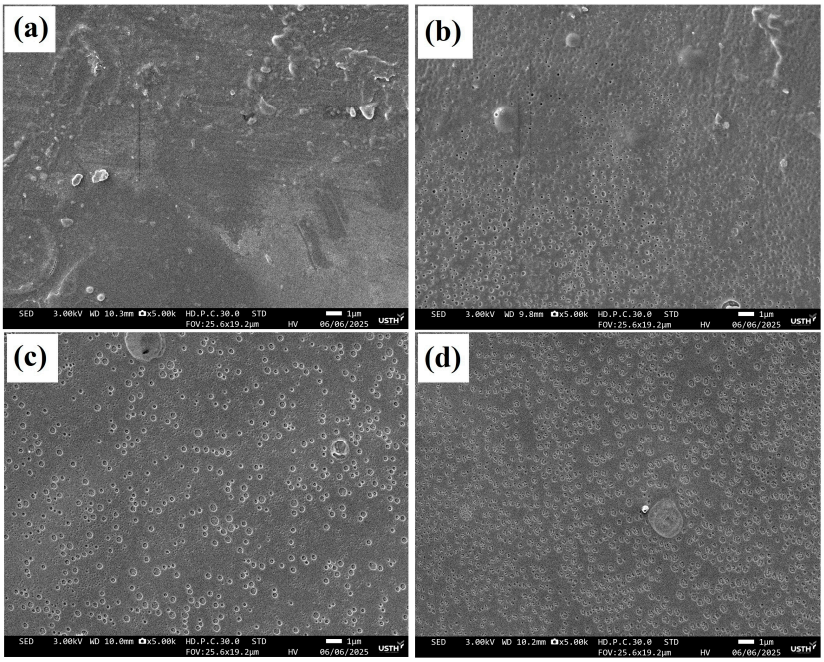


Figure 4. SEM images of the surface of acrylic-based coatings: (**a**)—control coating without extract, (**b**)—coating with 0.5 wt.%, (**c**)—coating with 1 wt.%, and (**d**)—coating with 1.5 wt.% *P. betle* extract.

The results of the natural field test are presented in Figures 5 and 6. After 6 months of immersion, the coating sample containing 1.0 wt.% *P. betle* extract demonstrated a high antifouling efficiency (over 80%), comparable to the Cu-based biocide sample. No visible macrofouling was observed on its surface. In contrast, the control sample (without antifouling additives) exhibited signs of macrofouling, particularly along the sample edges. However, after 9 months of immersion, the antifouling performance of *P. betle*-based coatings declined significantly compared to the Cu_2O-based biocide sample. Only the coating containing 1 wt.% of *P. betle* extract maintained a relatively high antifouling efficiency (74%). In contrast, the efficiency of coatings containing 0.5 wt.% and 1.5 wt.% extract dropped below 50% (Figure 6), and macrofouling appeared on the surface (Figure 5). For the sample containing 0.5 wt.% extract, the reduced antifouling performance may be attributed to insufficient active compounds remaining in the coating matrix over time. In the case of the 1.5 wt.% sample, the high self-polishing rate may have led to an accelerated release of the active antifouling agents from the coating surface, causing a rapid decline in antifouling effectiveness. No delamination or cracking was observed on the surface of the coatings (Figure 5).

Several review articles have been published in recent years describing natural compounds isolated from various organisms with antifouling properties [44–46]. These reviews cover over 700 natural compounds and over 100 synthetic analogues identified and synthesized between 2004 and 2020. However, it should be noted that, to identify effective antifouling compounds, laboratory studies must be complemented by subsequent verification of antifouling performance under natural marine conditions. Thus, only a few of the 700 known compounds with antifouling properties have been tested under complete immersion in seawater when incorporated into coating formulations. Table 5 presents the literature sources that report data on natural seawater tests of the antifouling activity of natural compounds (or extracts) incorporated into coating formulations.

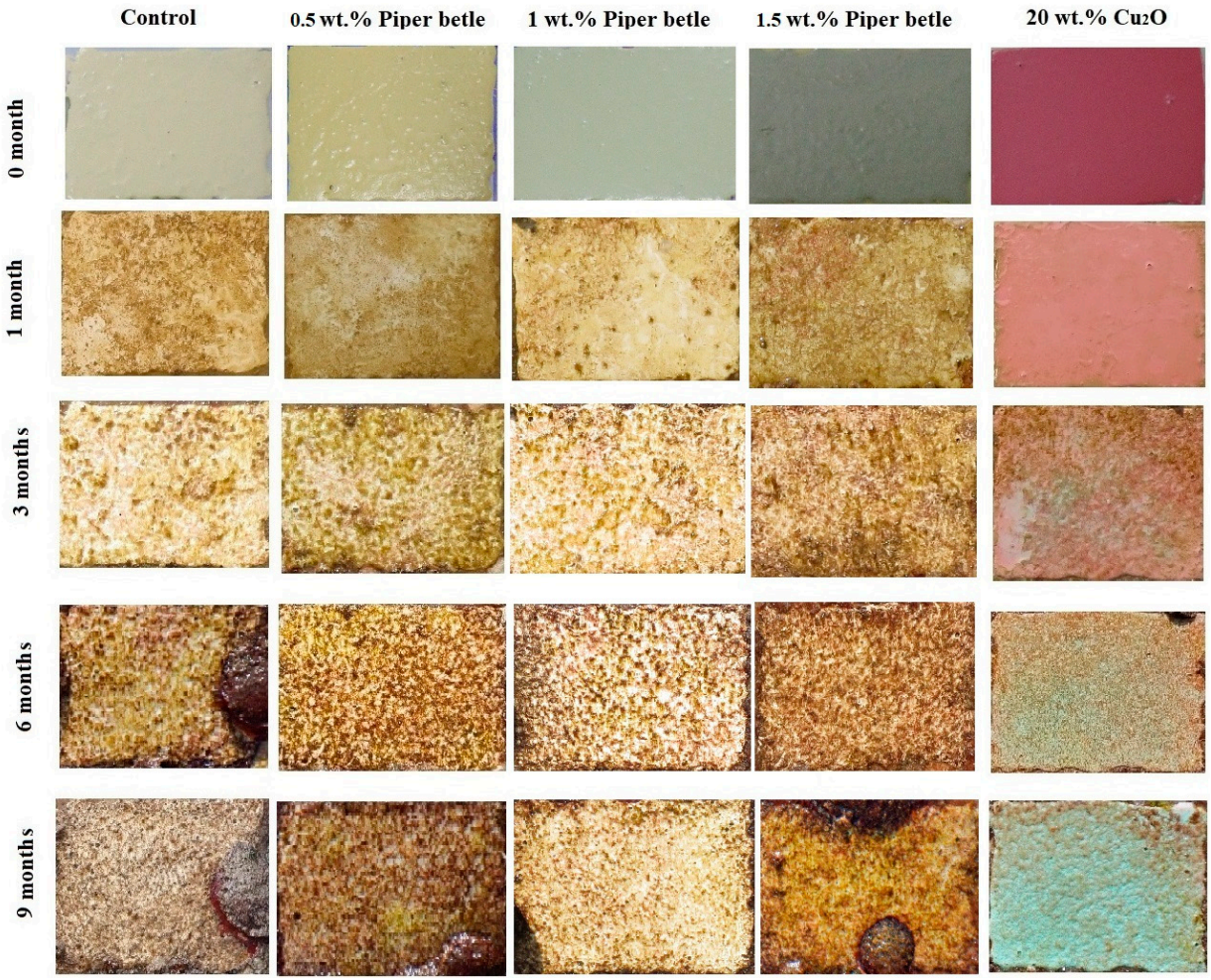


Figure 5. Visual observation of biofouling accumulation on coating surfaces during marine immersion.

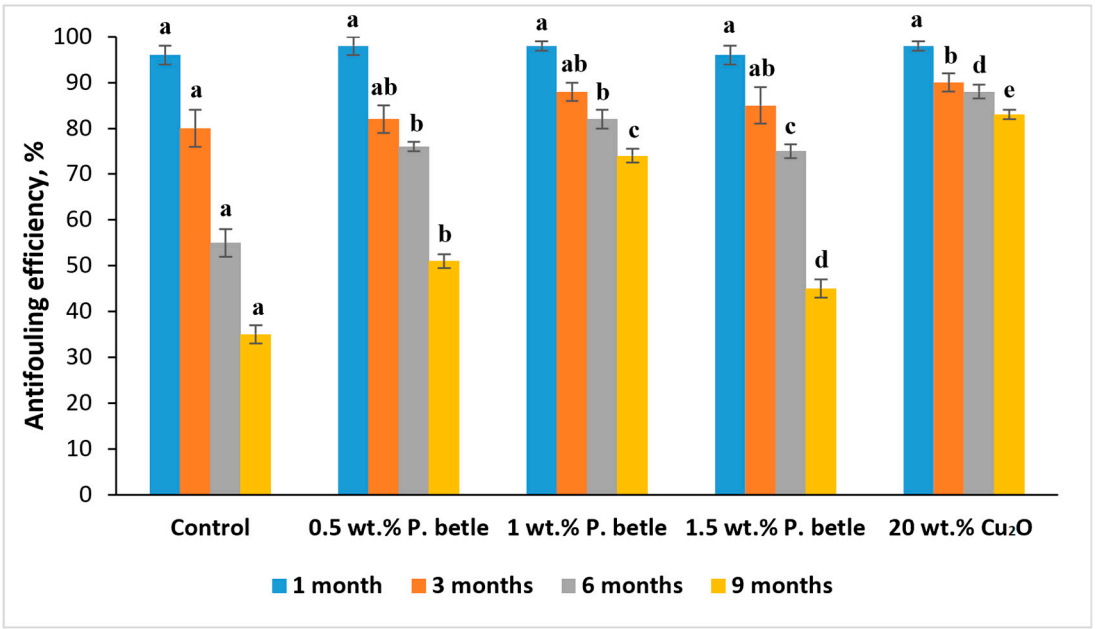


Figure 6. Antifouling efficiency of coatings after 9 months of immersion in Nha Trang Bay. Data are presented as mean ± SD ($n = 3$). Columns labeled with different letters indicate significant differences at $p < 0.05$.

Table 5. Some natural products incorporated into antifouling coatings.

Source of Natural Compounds	Test Duration and Location	References
Barettein and 8,9-dehydrobarettein were isolated from a marine sponge	2 months in seawater (near Sweden)	[47]
Tannins from plant extracts (chestnut, mimosa, quebracho)	4 months (Atlantic Ocean, near Argentina)	[48]
Rue (*Ruta graveolens*) and ginger (*Zingiber officinale*) extracts	6 months (Atlantic Ocean, near Brazil)	[49]
Plant alkaloid camptothecin from *Camptotheca acuminata*	11 months (near Dalipuyu Islet in Xiamen Bay)	[50]
Plant extract of *Nerium oleander*	1 month (Lingshui Bay, China)	[14]
Modified tannin derived from black acacia	7 months (Mediterranean Sea, Spain)	[51]
Tannins from mangrove trees (*Rhizophora apiculata*)	3 months (Chendering Port, Malaysia)	[52]
Plant extract of *Nardophyllum bryoides*	1.5 months (Mar del Plata harbor, Argentina)	[53]
Capsaicin and its derivatives from *Capsicum annuum*	3 months (Qingdao Bay, China)	[54]

One of the main limitations of antifouling coatings utilizing plant-derived bio-extracts is the difficulty in maintaining long-term antifouling efficacy. This issue is closely related to the inability to control the sustained release rate of the active antifouling agents from the coating surface. Moreover, the compatibility between the plant extract and the polymer matrix plays a crucial role in determining the durability of the coating under seawater immersion. To address this challenge, current research efforts have focused on several strategies, such as encapsulating the extracts into microcapsules [55] or modifying the coating matrix to regulate the release kinetics of the bioactive compounds [56].

4. Conclusions

This study investigated the composition and antibacterial activity of *P. betle* extract. The extract was incorporated into acrylic copolymer-based antifouling coatings to evaluate its potential as a natural bio-based additive. The results demonstrated that the *P. betle* extract contains high levels of bioactive compounds such as phenolics and flavonoids, exhibiting strong antibacterial properties. The extract showed significant antibacterial activity against representative bacterial strains and marine isolates. Incorporation of the ethanol extract of *P. betle* into the coating formulation at a concentration of 1 wt.% enhanced the antifouling performance of the coating under tropical marine conditions. These findings highlight *P. betle* extract as a sustainable alternative to copper-based agents and justify further work on controlled-release strategies to extend long-term performance.

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