

# Propolis extract as a green bacterial corrosion inhibitor on three types of metals

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**Abstract.** Watiniasih NL, Budiarsa IN, Antara ING, Wiradana PA. 2022. *Propolis extract as a green bacterial corrosion inhibitor on three types of metals. Biodiversitas 23: 4852-4860.* Corrosion causes severe damage to various types of metals, which is a serious problem in the infrastructure sector. Several studies have been conducted to find more efficient natural inhibitors. This study was aimed to determine the effectiveness of antioxidants from propolis extract in inhibiting the growth of bacteria that cause corrosion on various metals. The ethanol extract was carried out to obtain the bioactive contained in the propolis extract, then analyzed its antioxidant activity with DPPH and characterized using GC-MS. Various concentration of propolis extract were tested on NA+1% Fe medium to determine its resistance to corrosion-causing bacteria. Propolis extract was tested on several types of metals in inhibiting the corrosion rate. The results showed that the tested propolis extract had strong antioxidant activity with an IC<sub>50</sub> value of 82.496 ppm. The characterization of the metabolite profile showed that the propolis extract was dominated by chemical components that act as antioxidants and antibacterial agents. In the future, the results of this study can be used as a practical guide for the application of bioactive compounds through a biocontrol approach to the growth of corrosion-causing bacteria.

**Keywords:** Antioxidant, antimicrobial, corrosion, green-corrosion inhibitor, propolis extracts

## INTRODUCTION

Metals have an important role in supporting the progress of a nation's development and in the global economy (Zakeri et al. 2022). However, problems often occur especially when metallic materials come into contact with hydrogen atoms or molecules during the manufacturing, processing and service processes (Li et al. 2021). Hydrogen is able to be adsorbed onto the metal surface, therefore causes damage to mechanical properties and causes premature failure of the resulting steel structure (Liu et al. 2016). Corrosion is a natural process in which metals and their alloys attempt to return to a more stable thermodynamic state, as a result of a series of chemical processes or reactivity with the surrounding environment (Li et al. 2021; Prestat and Thierry 2021; Shang and Zhu 2021; Tanwer and Shukla 2022).

Globally, corrosion is a huge problem and it is estimated that around USD 2.5 trillion is spent annually to address the problems associated with rust. Interestingly, this amount is around 3-4 percent of the Gross World Product (GWP) each year (Koch 2017). High humidity environment has potential to produce corrosive activity, because of dissolving gases, like O<sub>2</sub>, CO<sub>2</sub> and other minerals (Barker et al. 2018). For example, in the oil and natural gas industry, oilfield formation always contains high concentrations of chlorides, carbonates, sulfates, and dissolved gases, such as H<sub>2</sub>S and CO<sub>2</sub>, which react with pipelines, causing corrosion, so oil leaking to the

environment (Yin et al. 2020). Corrosion causes the pipe lifespan decreases, causing economic losses and environmental pollution (Wasim et al. 2018).

Corrosion has always been a major problem in declining productivity of an industry, and researchers have focused on overcoming this problem (Angst 2018). Efforts in reducing and preventing the impact of corrosion are believed to save some economic losses (Yang et al. 2016; Zhang et al. 2012). In addition to environmental or abiotic factors, corrosion can also be caused by biotic factors, known as "Microbial Influences Corrosion (MIC)", the main cause of leakage in pipes (Li et al. 2018). The process starts with cell adhesion to a wet iron surface and results in a biofilm composed of a polymeric matrix and various cell populations in microcolonies (Muhammad et al. 2020). Adsorption of macromolecules (proteins, polysaccharides, and humic acids) and micromolecules (fatty acids and lipids) on the metal surface initiates the creation of biofilms, which affects the physical and chemical properties of the metal (Rodríguez et al. 2021). The types and abilities of microbial metabolites that cause corrosion have not been comprehensively understood (Nikolova and Gutierrez 2020; Salgar-Chaparro et al. 2020). However, the most dominant corrosion-causing species encountered were sulfate-reducing bacteria, residing in complex microbial communities (Hussain et al. 2016; Souza et al. 2017).

A review on green corrosion inhibitors shows that biological agents must meet important requirements to be used as corrosion inhibitors, should containing

phytochemicals or isolated organic compounds containing ether atoms (N, O, P, and S) (Hashim et al. 2012). For example, corrosion inhibitors made from gum extract have good ability to act as corrosion inhibitors and are understood through computational technology for the mechanism of each component of the extract (Kumar et al. 2022). Natural antioxidants from green tea are very potential as corrosion inhibitors by increasing polarization resistance, reducing the corrosion rate of rebar, and being better than positive control (calcium nitrite inhibitors) (Pradipta et al. 2019). Nutmeg oil which was tested as a natural corrosion inhibitor on carbon steel showed good adsorption, which was predicted to occur due to the presence of sabinene and -pinene compounds (Abdallah et al. 2021).

Many Balinese local communities have used propolis as a prominent traditional medicinal ingredient and has recently gained a lot of interest due to its high economic value. Propolis has been studied has an ability preventing various disease agents including infectious bacteria in humans (Tyastuti et al. 2006), however, the potential of propolis as a corrosion inhibitor agent by corrosion-causing bacteria on various types of metals has not been reported. Findings of this study are expected to be able to provide insight of knowledge and as a platform in the development of a biotechnology products based on Indonesian biodiversity. The propolis has the potential due to contains of bioactive compounds, environmentally friendly, and can be applied as a corrosion inhibitor agent of corrosion-causing bacteria.

## MATERIALS AND METHODS

### Sample collection

The propolis sample was collected from stingless bees farmed by local farmers across Bali. The samples were collected from the beehive by opening the lid of the beehive that was made out of wood (Figure 1). The propolis collected were then kept in bottles which has been sterilized and transported to the Bioscience Laboratory of Udayana University, Bali. The bottled samples were placed in refrigeration with the temperature set at 4°C until further testing is carried out on antioxidant activity, analysis of

bioactive ingredients, and antimicrobials that cause corrosion (Afata et al. 2022).

### Sample preparation and extraction

The collected propolis samples were then prepared for extraction by maceration method. The sample used in the analysis is crude propolis which was previously collected directly from the Trigona bee nest. The propolis sample extraction process was based on the procedure of Bankova et al. (1999), in short, before extraction, 500 grams of propolis samples were frozen at -20°C. Then homogenized and mashed by grinding using a mortar. Extraction was carried out by maceration method using ethanol as the solvent. In this method, the propolis is mixed with ethanol in a sterile container with a ratio of 1:5 between crude propolis and ethanol solvent.

Solvent extraction was carried out at 80% v/v (ethanol/water mixture) and tightly closed using a black bottle and stored in a cool, dry and dark place and being shaken twice for two weeks. The thing to note in this process is that it is important to allow the alcohol molecule to interact with as much of the propolis compound as possible. After two weeks, the supernatant was filtered using a Whatman No. 1 filter paper and then evaporated using a vacuum rotary evaporator. The liquid extract obtained was then stored in a refrigerator at 4°C in an airtight container until it was ready to be used in the test (Grange and Davey 1990; Muli and Maingi 2007).

### Determination of antioxidant activity of propolis extract

The antioxidant activity of propolis was determined based on its ability to trap free radicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH) according to the procedure of Conte et al. (2022). In summary, 10µl of propolis samples were evaluated based on their reactivity with 50 µl of DPPH (Sigma-Aldrich Chemical, USA). The plate was then incubated for 30 minutes at room temperature in the dark. The absorbance value was read at a wavelength of 517 nm using a UV-Vis Spectrophotometer (Thermo Scientific, USA). The inhibitory concentration of propolis was used to reduce the absorbance of DPPH by 50% (IC<sub>50</sub>) which was calculated from the calibration curve determined by linear regression.



**Figure 1.** Stup of *Tetragonula laeviceps* where propolis was harvested for this study

Analysis of bioactive component from propolis extract was carried out using Gas Chromatography-Mass Spectrophotometry (GC-MS). To meet the standards for analysis, the tool is conditioned before testing. The gas used is Helium UUHP with a sample injection volume of 1  $\mu$ l. The library used is NIST 14, HP % MS column type with split injection technique. The GC-MS analysis is performed based on the predetermined temperature method, namely the temperature in the column which is maintained at 70°C for 3 minutes. Then it is reduced to a temperature of 10°C/min then the temperature is raised to 280°C for 5 minutes. The propolis extract sample is injected into the inlet of the GC device which will then be separated from the analyte which is then forwarded to the MS tool. The GC flame ionization detector (FID) will provide a Total Respond Chromatogram (TRC) and the MS will provide specific analysis results at each TRC point or peak. Each peak point indicates the type of compound contained in the propolis extract which was then adjusted according to the Library NIST Mass Spectrometry Reference Database 14 (Pobiega et al. 2019; Salleh et al. 2021).

#### Determination of antimicrobial activity of propolis extract

Analysis of the antimicrobial activity of propolis extract was carried out by applying it to three different metals, namely stainless steel, iron, and galvanized steel. The metals used were obtained from commercial market. The metals were rectangular in safe with the size of 10 cm<sup>2</sup>. Three plates of metals of each type were treated with one of the propolis extract concentration, as repetition. The concentration of propolis was determined to be 0%; 0.125%; 0.25%; 0.5%; 5%; and 10%. The propolis extract was prepared by dissolving in distilled water and adding 2 drops of Tween solution. The concentration of 0% propolis extract was used as control. Each of the test metal was dripped with 1 ml of water and homogenized using a sterile ose needle, so as to dissolve the iron-reducing bacterial suspension. Water was placed in a tube containing 4 ml of sterile water and vortexed to achieve a homogeneous mixture. Water is put into a tube containing 4 ml of sterile water and vortexed so that it is homogeneous.

Five hundred  $\mu$ l of suspension was taken into a sterile tube containing 4,500  $\mu$ l of sterile water as a dilution of 102. Then 500  $\mu$ l of suspension was taken and put into a Petri dish previously filled with Nutrient Agar (NA) + 1% Fe media. The suspensions that have been planted were incubated at 37°C for 48 hours. After the incubation period, the growth of colonies on each media was observed through colony formation. Each colony that grows is identified by morphological characters, then identified based on the differences in morphological characters that have been seen (Kurniawan et al. 2022 a, b; Sani et al. 2020).

Bacteria that are able to grow on 1% NA + Fe media are bacteria that are able to reduce metals for their metabolic needs and subsequently have the potential to

cause metal rust. The number of bacterial colonies growing in the growth medium was calculated by the total plate count (TPC) method. The number of bacteria counted showed the inhibitory ability of propolis extract on the growth of iron-reducing bacteria. The effect of giving propolis extract on the total number of bacteria was then plotted in a graph to determine the trend of its effectiveness.

#### Data analysis

Statistical analysis was performed using SPSS 22 software (IBM, USA) with Two-Way ANOVA between the effect of different concentrations of propolis extract on the total numbers of bacteria (CFU/ml) on the three test metals. To determine the difference between the propolis extract treatments, the Tukey HSD test was continued with a significance level of 0.05 ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

#### Antioxidant activity of propolis

Propolis exerts strong antioxidant activity as evidenced by its ability to reduce DPPH in low concentrations ( $IC_{50} = 82.5$  ppm) (Table 1). The research finding is in line with previous studies that determined the antioxidant activity of propolis extract collected from beekeepers in Brazil which showed an  $IC_{50}$  value of 18.52 ppm (Conte et al. 2022). Propolis found at Middle of Delta, Egypt which was extracted using ethanol solvent was reported to have higher antioxidant activity when compared to aqueous extracts of 94.45%  $\pm$  0.85 and 90.01%  $\pm$  0.18 (Ibrahim and Alqurashi 2022). Likewise, propolis from the stingless bee, *Tetragonula sapiens* from South Sulawesi, Indonesia has an  $IC_{50}$  value of 9.694 ppm which indicates very strong antioxidant activity (Farida et al. 2022).

The antioxidant activity categories were grouped into the following classifications: strong ( $IC_{50} < 50$  ppm), moderately strong ( $IC_{50}$  50 - 100 ppm), moderate ( $IC_{50}$  101 - 250 ppm), weak ( $IC_{50}$  250 - 500 ppm), and very weak ( $IC_{50} > 500$  ppm) (Jun et al. 2003). The DPPH test works based on the reaction of the DPPH radical with a hydrogen donor molecule from propolis extract. The content of bioactivity contained in propolis extract inhibits the rate of oxidation of other molecules that depend on their concentration and reactivity to reactive oxygen species (Kocot et al. 2018). A lower  $IC_{50}$  value correlates with a higher DPPH radical scavenging activity which represents the concentration of extract required to reduce by 50% of the initial absorbance of DPPH solution (Sukweenadhi et al. 2020; Loganayaki et al. 2013; Rahman et al. 2015).

In general, compounds that have phenolic characters, including substances capable of expressing the ability to scavenge free radicals, were also found in propolis extracts from bees (Mohdaly et al. 2015; Nichitot et al. 2021). These compounds consist of several components of metabolites such as flavonoids, phenylpropanoids, terpenes, stilbenes, lignins, coumarins, and their prenylated

derivatives. It should be noted that the composition of these metabolites varies according to the geographical location and botanical origin used as a source of pollen (Huang et al. 2014; Popova et al. 2021). Recently, attention has begun to develop towards compounds whose metabolites contained in propolis extracts that allow the use of such compound for the prevention of oxidation processes and the formation of ROS as well as chelating pro-oxidative metals (De Paula et al. 2017).

### Bioactive compounds of propolis

According to the findings, the chemical composition of propolis extract obtained from beekeepers in Bali Province included up to 16 bioactive components. Sorbitol, 1-tetradecanamine, and erythritol are examples of antibacterial chemicals. Dodecyltrimethylammonium bromide, formamide, and oxalic acid, on the other hand, stop corrosion. Propanoic acid and furanone, on the other hand, stop corrosion and kill bacteria, respectively (Table 2).

Previous studies of GC-MS analysis of propolis extract also reported similar results which revealed compounds such as sugars, carboxylic acids, terpenoids, sugar alcohols, hydrocarbons, aldehydes and amino acids. The main compound found in the propolis extract was sugar which is around 31.4% (Salleh et al. 2021). Some types of reducing sugars include ribose, fructose, glucose, and galactose. As is the case with honey collected from bees in Malaysia, it shows very high content of fructose and glucose which is

associated with its preference for various types of inflorescences utilized by honey bees (Cheng et al. 2019).

In this findings, sorbitol is one of the sugar alcohols found in propolis extract which is generally used as a natural sweetener and preservative in food products to prevent the growth of bacteria that cause food spoilage. Sorbitol, also known as d-glucitol, is a six-carbon sugar alcohol naturally found in many fruits (Zarour et al. 2017). Previous studies mentioned the ability of sorbitol to have the ability as an antimicrobial for oral health diseases (Kim et al. 2016). Sorbitol-coated HSSP (SC-HSSP) is highly antimicrobial which is used as a disinfectant for the treatment of fresh vegetable products by reducing the total aerobic and coliform bacterial population (Tsuruma et al. 2020).

**Table 1.** Antioxidant activity of bee propolis extract collected from Bali Province, Indonesia

No	Concentration	Abs. (516.0 nm)	Scavenging
1	Control	0.726	***
2	60 ppm	0.492	32.190
3	70 ppm	0.431	40.550
4	80 ppm	0.386	46.790
5	90 ppm	0.311	57.162
6	100 ppm	0.264	63.533

Note: Correlation coefficient of scavenging and concentration for IC<sub>50</sub> value in propolis extract. Y = 0.793x - 15,42; R<sup>2</sup> = 0.994; IC<sub>50</sub> = 82.49 ppm.

**Table 2.** Profile of propolis extract bioactive compounds based on GC-MS analysis

No.	Retention time	Peak area (%)	Chemical compound	Role	Reference
1	2.797	1.35	Oxalic acid	Corrosion inhibitor on copper	Rashid and Khadom (2022)
2	2.926	1.63	2,3 butanediol (BDO)	Antibacterial, Feed additives, and chemical and drugs development	Li et al. (2022); Narisetty et al. (2022); Rashid and Khadom (2022)
3	3.229	1.67	Formamide	Antibacterial and corrosion protective agent	Alam et al. (2020)
4	3.595	3.44	Glyceraldehyde	Antibacterial	Ong et al. (2019)
5	4.381	1.49	Butanal	Synthetic resins, plasticizers, agrochemicals, and antioxidants	Klein (2014)
6	4.487	6.65	Furanone	Antibacterial, food additives, pheromone and anti-carcinogenic	Slaughter 1999)
7	5.061	5.26	Jasmone	Repellent, natural herbicide	Vieira et al. (2013)
8	6.064	0.38	Erythritol	Antibacterials	de Cock (2018)
9	10.227	1.53	5-hydroxymethylfurfural	Antioxidant	Essien et al. (2021); Kowalski (2013); C.-H. Lee et al. (2020)
10	10.473	1.86	Propanoic acid	Antioxidant, corrosion inhibitors	Abd-Elal et al. (2018); Lee et al. (2007)
11	13.585	9.24	5-hydroxy-4-methyl-3-heptanone	Natural pheromones	Mori and Ebata 1986)
12	14.017	3.12	Dodecyl trimethyl ammonium bromide	Anti-corrosion on steel	Yin et al. (2020)
13	15.544	7.17	1-arabinopyranoside	Antioxidant	Vera Saltos et al. (2015)
14	16.377	1.04	1-tetradecanamine	Antioxidants and biosurfactant	Abdelsattar et al. (2022)
15	19.181	16.87	Sorbitol	Antifungal, natural plasticizers, biosensors	Gessei et al. (2022); Sukkaneewat et al. (2022)
16	25.700	22.14	Di-n-octyl-phthalate	Biofuel, plasticizer	Lodi et al. (2022); Zheng et al.

The Compound 1-tetradecanamine is an amino acid oxide that acts as a surfactant and is known to have the ability as an antibacterial agent such as *Escherichia coli* and *Staphylococcus aureus* (Birnie et al. 2000). Erythritol compounds also have antimicrobial effects through inhibition of the growth of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Actinomyces viscosus*. The cytotoxic effect of erythritol powder is also very clear when applied in vitro to human gingival fibroblasts (HGF) which is able to influence the processes of cell viability and cell proliferation well (Weusmann et al. 2022). In its role as a corrosion inhibitor, the bioactive compound of propolis is mostly played by Dodecyltrimethylammonium bromide, especially in the form of propanediyl-1,3-bis (N,N dimethyl-N-dodecylammonium bromide (PDDB), which clearly increases the inhibitory efficiency with increasing concentration (Yin et al. 2020). This PDDB compound works optimally when mixed together with propanediyl-1,3-bis (N,N-dihydroxyethyl-N-dodecylammonium bromide (PDHDB) so as to form a protective layer on the carbon steel surface which further causes inhibition. corrosion rate (Odewunmi et al. 2020).

Oxalic acid (OA) can generally be found naturally in various plants, animals, and microorganisms (Zafar et al. 2022). In some findings, the addition of oxalic acid was able to affect the inhibition of several types of metals including aluminum-magnesium (Rashid and Khadom 2022) and copper (Bernard et al. 2007). Furthermore, propanoic acid compounds are important compounds in the formation of non-ionic surfactants in inhibiting or reducing steel corrosion in corrosive environments at low concentrations (Bedir et al. 2021). Interestingly, the presence of functional groups in propanoic acid such as hydroxyl groups, ethylene oxide, carbonyl, double bonds, benzene rings, oxygen atoms, nitrogen atoms in addition to the hydrophobic chain of the surfactant is responsible for increasing its adsorption ability on the steel surface (Abd-Elal et al. 2018). Furanon compounds have the potential to inhibit microbial corrosion associated with Gram-positive bacteria (Srinivasan et al. 2021; Wu et al. 2012). Based on previous research, it was found that the addition of a concentration of 40 mg/ml furanone was able to inhibit the growth of *Desulfotomaculum orientis* (a microbe that causes corrosion) by 96% (Ren et al. 2001). This makes furanone a potential inhibitor of corrosion-causing bacteria.

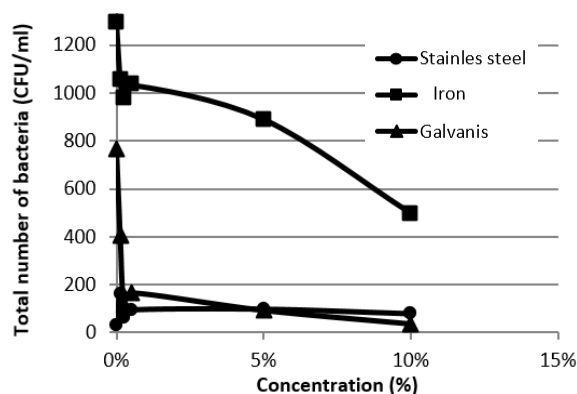
The mechanism of protection of natural materials against iron or steel from corrosion attack is estimated to be almost similar to the mechanism of protection by organic inhibitors. The reaction that occurs between  $\text{Fe}^{2+}$  metal and a corrosive medium such as  $\text{CO}_2$  is estimated to produce  $\text{FeCO}_3$ . Further oxidation produces  $\text{Fe}_2(\text{CO}_3)_3$  and the reaction between  $\text{Fe}^{2+}$  with inhibitors of natural extracts produces complex compounds, including propolis extract. On the other hand, inhibitors of natural extracts containing nitrogen donate a pair of electrons to the surface of the mild steel metal when  $\text{Fe}^{2+}$  ions diffuse into the electrolyte solution. The reaction becomes  $\text{Fe} \rightarrow \text{Fe}^{2+} + 2\text{e}^-$  (releasing

electrons) and  $\text{Fe}^{2+} + 2\text{e}^- \rightarrow \text{Fe}$  (accepting electrons). The product formed above has high stability compared to Fe alone, so samples of iron or steel that are given natural extract inhibitors will be more protected against corrosion (Pradityana et al. 2016).

### Total bacteria number in metals

The application of propolis extract to three types of metal in this study (stainless steel, iron, and galvanized) with varying concentrations and incubated for 12 weeks in an open room and room temperature showed variations in the total number of bacteria. The worst corrosion rate was found in iron at all concentrations of propolis extract when compared to stainless steel and galvanized. Bacteria grown on petri dish added with Nutrient agar + 1% Fe medium showed the highest number of bacteria in ferrous metals compared to the other two types of metals. We assume that the number of bacteria that can survive on metals may be responsible for the severity of corrosion in each metal, especially bacteria that are able to reduce iron. Generally, iron-reducing bacteria will be able to take advantage of the Fe content as the final electron acceptor and then reduce it to reddish rust that sticks to the metal. On the other hand, the low number of bacteria in stainless steel and galvanic was in line with the low rust appearance, indicating that propolis extract was effective as a corrosion inhibitor for these two metals, but not for iron.

The findings may occur because abiotic factors such as oxygen, water content, and humidity of the room where this experiment was carried out. However, it should be noted that the propolis extract in this research was able to act as an antimicrobial agent by reducing the presence of iron-reducing bacteria which in turn is able to inhibit the corrosion process of the test metal. The curve of the total number of bacteria on the metal against variations in the concentration of propolis extract is shown in the following Figure 2.



**Figure 2.** The total numbers of bacteria on stainless steel, iron, and galvanized metal added with variations in the concentration of propolis extract in vitro

**Table 3.** The average result of the effect of variations in the concentration of propolis extract on the total number of bacteria grown on Nutrient Agar (NA) + 1% Fe medium

Tukey HSD <sup>a,b</sup>	
Concentration (%)	Mean
0	699.7778 <sup>a</sup>
0.13	546.8889 <sup>b</sup>
0.25	417.2222 <sup>c</sup>
0.50	439.2222 <sup>d</sup>
5	361.9444 <sup>e</sup>
10	210.6667 <sup>f</sup>

Note: the difference in notation in the same column showed a significant difference ( $p < 0.05$ ) based on the Tukey HSD test.

**Table 4.** The average result of the effect of propolis extract on the number of bacteria on the three types of metal tested

Metals	Mean
Stainless steel	96.9444 <sup>a</sup>
Iron	273.3056 <sup>b</sup>
Galvanized	967.6111 <sup>c</sup>

Note: the difference in notation in the same column showed a significant difference ( $p < 0.05$ ) based on the Tukey HSD test.

Based on previous research, it was known that the addition of propolis ethanol extract was able to provide inhibition of the corrosion process on SAE 1010 carbon steel which was measured qualitatively and quantitatively (Rizvi et al. 2020). Research conducted by Fouda and Badr, (2013) also revealed the potential of propolis extract as a corrosion inhibitor in carbon steel. These findings further explain the ability of propolis aqueous extract as an effective inhibitor agent for the corrosion of carbon steel in aqueous media. On the other hand, based on statistical tests on the effect of the concentration of propolis extract on the total number of bacteria that grew, there was a significant difference ( $p < 0.05$ ). These results mean that each increase in the concentration of propolis extract can cause a significant decrease in the total number of bacteria (Table 3). This significant reduction in bacteria also allows for a decrease in the corrosion rate of the metal-containing media in this study. Likewise, the effect of propolis extract on the number of bacteria was significantly different for the three metals (Table 4). The highest mean of the effect of propolis extract successively occurred in Galvanized metal, iron, and stainless steel.

Other findings that apply biological agents as corrosion inhibitors such as *Artemisia argyi* (ALE) leaf extract which is reported to be able to slow down the corrosion process of carbon steel in HCl with a maximum inhibitory efficiency of 96.4% as well as the inhibition mechanism is proven by the results of quantum calculations and molecular dynamics simulations (Wang et al. 2022). Cabbage extract has excellent performance to inhibit corrosion of X70 steel in HCl solution with its inhibition efficiency reaching 95.37% (Sun et al. 2022). A new application of agroindustry waste utilization as an environmentally-based corrosion inhibitor agent from the pyrolysis of *Sygarus*

*coronata* is reported to be efficient in inhibiting corrosion up to 91% through gravimetric and electrochemical measurements (Fernandes et al. 2022). However, our findings may be the first to report the effectiveness of propolis extract in inhibiting the growth of corrosion-causing bacteria applied directly to all three metals.

In conclusion of this study is that propolis extracts from Trigona bees from various locations in Bali Province has a strong antioxidant activity because of the content of various profiles of bioactivity compounds. In vitro, propolis extracts was able to inhibit the growth of bacteria that cause corrosion on NA + 1% Fe media which depended on the increasing percentage of extract concentration. Testing of extracts on three types of metals showed that propolis extract had the ability to inhibit the highest corrosion rate in the Galvanized type in this study. This propolis extract can be absorbed on the surface of the substrate and form a protective layer on the test metal from corrosion-causing bacteria.

Finally, further research is needed to confirm the types of bacteria that cause corrosion on metals by using the DNA Barcoding approach so that it can accurately provide comprehensive information to relevant authorities in making public policies in an effort to apply green inhibitor agents such as propolis extract. Likewise, further identification of certain bioactive components that play a role in corrosion inhibition still needs to be done in the application of organic Green Corrosion Inhibitors (OGCIs) in the future.

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