Research Article

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Antibacterial epoxy composites with addition of natural Artemisia annua waste

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Abstract: Antibacterial epoxy resins (EP) have great potential in medical and electronic fields. During the process of extracting artemisinin from Artemisia annua, artemisia naphtha (AN) is generated as waste. The components of AN show antibacterial activity, and hence, it is introduced as a novel antibacterial agent in the epoxy matrix. In this study, the properties of epoxy resins with various AN loading were investigated. The results showed that AN/EP composites presented strong antibacterial activity against Escherichia coli and Staphylococcus aureus at the sterilization ratio of 100% against E. coli and 99.96% against S. aureus, respectively. Meanwhile, the thermal properties (curing temperature and glass transition temperature) of AN/EP composites remained well, and the mechanical property was even improved. Especially, the flexural strength of AN/EP composites could be reinforced by 62.9% when the content of AN was up to 5 wt%. For comparison, Artemisia annua powder (AAP), which was directly smashed from natural A. annua, was also mixed with epoxy resins as an antibacterial agent and showed excellent antibacterial property. Therefore, antibacterial epoxy composites containing A. annua waste as a natural resource with the enhanced mechanical property may have enormous potential in future biological and healthcare fields.

Keywords: artemisia naphtha (AN), Artemisia annua powder (AAP), epoxy resins, antibacterial, Artemisia annua waste

1 Introduction

Due to the distinguished mechanical, thermal, and dielectric properties, epoxy resins (EPs) are widely used in many areas such as coatings, reinforced composites, casting, adhesives, pouring sealants, and other industries (1,2). Nevertheless, the service life of epoxy resins would be shortened by bacteria over time. Recently, the protection of epoxy resins against microbial contamination has aroused much attention (3).

To improve the antibacterial property of epoxy resins, two common methods are used through changing the internal chemical structure and adding antibacterial agents. Although Modjinou et al. (4) designed bio-based crosslinked co-networks from resorcinol diglycidyl ether and a eugenol derivative to improve antibacterial properties, it is still challenging to graft antibacterial functional groups such as phenolic hydroxyl group and nitrogen group (5). Hence, adding antibacterial agents is a more effective method. Inorganic antibacterial nanoparticles have been studied and reported, such as silver nanoparticles (6), zinc oxide nanoparticles (7,8), cuprous oxide nanoparticles (9), and titanium dioxide nanoparticles (10,11). However, the high surface area of nanoparticles and their poor compatibility with polymer could lead to the aggregation of nanoparticles and the poor properties of polymer (12–14). Compared to inorganic antibacterial agents, organics, with chemical groups similar to polymers, are more compatible with epoxy resins. For cost reduction, deriving organic antibacterial agents from renewable resources such as natural products has received widespread attention (15). Kohsari et al. (16) prepared a chitosan–polyethylene oxide (CS–PEO) nanofibrous mats preferred for biomedical applications with more than 99% antibacterial activity. Rajabali and Molod (17) studied the controlled release of ciprofloxacin as an antibacterial additive by the hydrogel and found that the hydrogel could act as an antibacterial drug carrier, but the antibacterial activity of the hydrogel is closely related with the pH condition that would be limited in practical application. Zavareh et al. (18) obtained an epoxy-based hybrid material with antimicrobial activity by adding a natural modifier oregano essential oil (OEO) content up to
15 wt%, while their strength and modulus decrease with the addition of OEO. Although good antibacterial property in epoxy resin can be achieved by adding a large amount of organic antibacterial fillers, it would lead to the reduction in other properties of epoxy resins, such as mechanical property.

_Artemisia annua_ is a natural perennial herb used as a traditional Chinese medicine, whose leaves are mainly used to extract artemisinin (A) being used against _Plasmodium falciparum_ malaria due to a unique chemical peroxide bridge of the sesquiterpene lactone (19–21). The antimalarial effect of artemisinin also indicates certain antibacterial activity. Further studies have also confirmed that the slag powder and water decoction of artemisinin have a strong bacteriostatic effect on _Anthrax bacillus, Staphylococcus epidermidis, Catarrh_, and _Diphtheria bacillus_ and have a certain bacteriostatic effect on _Tuberculosis bacillus, Pseudomonas aeruginosa, Staphylococcus aureus, Dysentery bacillus_, and so on (22,23). However, while extracting artemisinin from _A. annua_, only small amount of artemisinin (approximately 0.1–0.7%) (24) can be obtained, and other components such as artemisia naphtha (AN) are abandoned, which cause a waste of resources. Therefore, to utilize the waste resources such as AN is very important. The researchers have found that _A. annua_ powder (AAP), which is directly smashed from _A. annua_, can be divided into volatile components (AN) and nonvolatile components (including artemisinin and others). As a kind of volatile component, AN is mainly composed of terpenoid or phenolic hydroxyl functional groups like eucalyptol, terpinen-4-ol, eugenol, artemisia ketone, and beta-pinene (Figure 1) (25–27). Abundant terpenes (28) and phenolic hydroxyl groups (29) in these components may have antimicrobial activity. By using AN as an antimicrobial agent, a new route for polymer antimicrobial can be opened. In addition, as AAP contains AN and artemisinin, AAP also suggests the antibacterial activity, which can be also used as a promising antibacterial agent.

The components of AN are full of polar group, which suggests the strong interaction with polar epoxy resins. For example, the OH groups in terpinen-4-ol and alpha-terpinylpropionate can react with epoxy, resulting in the ring-opening reaction, and some other polar groups like the epoxide group in eucalyptol may dissolve in the epoxy matrix owing to the similar group with epoxy resins (Figure 1). The interaction between AN and epoxy resins may optimize their compatibility and thus enhance mechanical properties of epoxy resins. Besides, AAP can also interact with epoxy resins as it contains AN. Hence, AN and AAP are possible to be introduced into epoxy resins.

Considering rich potential of antibacterial compositions in AN, the aim of the present work is to develop a sustainable antibacterial epoxy composite using _A. annua_ waste for the first time. AN extracted by steam distillation from natural _A. annua_ waste was mixed with epoxy resins in varying loading levels from 1 to 7 wt%. The antibacterial property, morphology, flexural strength, curing temperature, and transition of glass

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Figure 1: The main components of AAP and some chemical structures of components in AN.
temperature of AN/EP were studied in detail. For comparison, pure artemisinin and AAP smashed directly from its leaves are also used as antibacterial agents, and their properties have been investigated.

## 2 Material and methods

### 2.1 Materials

A mixture of epoxy monomers: 201G ((3,4-epoxy cyclohexyl methyl 3,4-epoxy cyclohexyl formate (equivalent: 150-17Gm/Eq, CAS: 2386-87-0) and diglycidyl 1,2-cyclohexanedicarboxylate (equivalent: 135 Gm/Eq, CAS: 5493-45-8)) and curing agent MHHPA (methylhexahydrophthalic anhydride, CAS: 25550-51-0) were purchased from Shanghai Resin Factory Co. Ltd, China. Artemisinin, AN, and A. annua powder (AAP) were received from Xi’an Xin Lu Biological Technology Co. Ltd, China. AN was extracted by steam distillation (30), and AAP was directly smashed from A. annua leaves by grinding.

### 2.2 Preparation of A/EP, AN/EP, and AAP/EP composites

Five grams of 201G epoxy was mixed with 5 g curing agent MHHPA (w/w = 1/1) and stirred for 10 min in a three-dimensional high-speed mixer (ksh-100, China) and then degassed by a vacuum pump to remove air bubbles. Then, the mixture was poured into a polytetrafluoroethylene mold and cured at 160°C for 2 h in a vacuum drying oven (DZF-6050, China).

For the preparation of AN/EP composites, different quantities of AN from 1 to 7 wt% were added into 5 g 201G at room temperature as given in Table 1 (AN/EP1, AN/EP3, AN/EP5, and AN/EP7). Then, the mixture was constantly stirred for 10 min until AN was completely dissolved. After that, 5 g MHHPA was added and mixed for another 10 min. The mixture was degassed, poured into a polytetrafluoroethylene mold, and cured at 160°C for 2 h.

For the preparation of AAP/EP composites, AAP was first dried at 80°C for 4 h. Different quantities of AAP (from 1 to 7 wt%) were added into 5 g 201G at room temperature as given in Table 1 (AAP/EP1, AAP/EP3, AAP/EP5, and AAP/EP7). The mixture was stirred for 10 min until homogeneous dispersion was obtained. Then, 5 g MHHPA was added and mixed for another 10 min. The mixture was degassed, poured into a polytetrafluoroethylene mold, and cured at 160°C for 2 h.

For comparison, A/EP composite with 5 wt% pure artemisinin (A/EP5) was prepared in a similar condition.

### 2.3 Characterization

#### 2.3.1 Antibacterial activity

The antibacterial activity on the surfaces of nanocomposites was evaluated against Gram-negative E. coli and Gram-positive S. aureus. According to GB/T 21510-2008 (31), bacteria were sucked and blew by 3.0–5.0 ml of phosphate buffer (0.03 mol/l, pH = 7.2–7.4) into agar culture medium and then mixed using the oscillator for 4 h. A total of 100 μL of bacterial suspension was dropped on the blank sample and tested samples. Then, the samples were smoothly covered with plastic wrap without bubbles in duplicate and incubated at 37 ± 1°C and 90% relative humidity levels for 24 h. After that, the eluant and its 10-fold serial dilution in phosphate-buffered saline (PBS) as a parallel test was performed for

| Samples | 201G (g) | MHHPA (g) | Artemisia naphtha (g) | Artemisia annua powder (g) | Artemisinin (g) |
|---------|---------|----------|----------------------|--------------------------|----------------|
| EP      | 5.00    | 5.00     | 0.00                 | —                        | —              |
| AN/EP1  | 5.00    | 5.00     | 0.10                 | —                        | —              |
| AN/EP3  | 5.00    | 5.00     | 0.31                 | —                        | —              |
| AN/EP5  | 5.00    | 5.00     | 0.53                 | —                        | —              |
| AN/EP7  | 5.00    | 5.00     | 0.75                 | —                        | —              |
| AAP/EP1 | 5.00    | 5.00     | —                    | 0.10                     | —              |
| AAP/EP3 | 5.00    | 5.00     | —                    | 0.31                     | —              |
| AAP/EP5 | 5.00    | 5.00     | —                    | 0.53                     | —              |
| AAP/EP7 | 5.00    | 5.00     | —                    | 0.75                     | —              |
| A/EP5   | 5.00    | 5.00     | —                    | —                        | 0.53           |
the bacteria of the 10 ml suspension recovered from the test samples. Then, 200 μl of each dilution was put into the sterilized agar culture medium in duplicate and incubated at 37 ± 1°C for 24 h. After incubation, the number of colonies of each dilution was counted, and the mean values of the counts were reported. The sterilization ratio could be calculated using the following equation:

\[ R = \frac{\beta_0 - \beta_s}{\beta_0} \times 100\% \]  

(1)

where \( R \) is the sterilization ratio, \( \beta_0 \) is the number of bacterial colonies for the blank sample, and \( \beta_s \) is the number of bacterial colonies for each test sample. For each test, four parallel samples were prepared.

### 2.3.2 Scanning electron microscope (SEM)

Epoxy, AN/EP5, and AAP/EP5 composites were soaked in liquid nitrogen for 2 h and then broken. The cross-sectional morphologies of surface fracture were measured using an environmental scanning electron microscope, model Quanta 250 FEG (field Emission Gun), FEI Company, Netherlands. This instrument was attached to an EDX unit (Energy dispersive X-ray analyses). The maximum accelerating voltage was 30 kV, and the full magnification range was 14× up to 10,000×. Before insertion into the chamber and observation under the SEM instrument, the samples were sputter coated using an EMITECH sputter coater, model K550X, England. The SEM electron micrographs were recorded at 20 kV.

### 2.3.3 Mechanical tests

The flexural tests for samples were conducted on a universal electromechanical tester (Model Instron 4465, USA) at room temperature according to ASTM D790-2007 with a tensile speed of 2 mm min\(^{-1}\) (32). All the flexural data were collected based on three samples.

### 2.3.4 Thermal measurement

Differential scanning calorimetry (DSC) (Q-2000, TA, USA) was used to study the influence of AN and AAP on the curing temperature and glass transition temperature of epoxy resins. Approximately 5 mg of the sample before curing was placed in a solid crucible and heated in the nitrogen atmosphere from 40 to 200°C at a heating rate of 10°C min\(^{-1}\) to study curing temperature. Besides, approximately 10 mg of the sample after curing was placed in a solid crucible and heated in the nitrogen atmosphere from 40 to 200°C at a heating rate of 10°C min\(^{-1}\) to measure glass transition temperature.

The thermogravimetric analysis (TGA) was conducted on a PerkinElmer TGA1 thermogravimetric analyzer (TGAQ-5000, TA, USA). Approximately 5 mg of artemisinin, EP, AN, and AAP samples were heated in the nitrogen atmosphere from 40 to 700°C at a heating rate of 10°C min\(^{-1}\).

## 3 Results and discussion

To study the feasibility of compounding artemisinin, AN, and AAP with EP, TGA was studied, as shown in Figure 2. As the curing temperature of EP during the process was around 160.0°C, all the antibacterial agents should remain stable under this temperature. From Figure 2, the initial decomposition temperature of AN, AAP, and artemisinin was above 181.4°C, so the results indicate that they are stable in the epoxy matrix at high temperature and could maintain the antibacterial effect after being compounding with epoxy. Therefore, artemisinin, AN, and AAP could be added into epoxy resins for further study.

### 3.1 Antibacterial property

In this study, both *E. coli* and *S. aureus* were studied. Meanwhile, the same sample with bacteria was diluted...
10-folds and retested for another two times, providing the similar results that proved a relatively stable antibacterial performance. The pure epoxy revealed a dense bacterial colony (Figure 3a and d) with E. coli and S. aureus, respectively, while epoxy composites containing 5 wt% AN showed good antibacterial activity with no obvious bacterial colony (Figure 3b and e). Therefore, AN may have a significant impact on the antibacterial activity of EP, and thus, the specific sterilization ratios of epoxy composites containing different contents of AN were further studied.

The sterilization ratios of epoxy composites with different contents of AN and AAP against E. coli and S. aureus were investigated as shown in Figure 3c and f, respectively. It showed that pure epoxy had little antibacterial activity. With the increasing content of AN, the AN/EP composites revealed a rapid increase of sterilization. The sterilization ratio of the epoxy composite containing 3 wt% AN against E. coli was about 97.74%, while that containing more than 5 wt% AN was up to 100% as shown in Figure 3c. Besides, the AAP/EP composites showed similar antibacterial activity trends. The sterilization ratio of AAP/EP5 was up to 100% against E. coli, which was not less efficient than AN/EP5 and A/EP5. According to Zavareh et al. (18), with the content of 15% addition of natural antibacterial agents, the EP reached approximately antibacterial saturation, while the significant improvement in the antibacterial activity of these EP composites can be obtained when a lower antibacterial agent content (no matter AN, AAP or artemisinin) of 5 wt% was added.

In addition to Gram-negative bacillus, the similar trends appeared in AN/EP composites against Gram-positive S. aureus. Analogously, the sterilization ratio boosted with the increasing content of AN, and epoxy composite containing 5 wt% AN showed the most

![Figure 3: Antibacterial activity of (a) pure EP and (b) AN/EP5 against E. coli; (c) the sterilization ratio of composites against E. coli; antibacterial activity of (d) pure EP and (e) AN/EP5 against S. aureus; (f) the sterilization ratio of composites against S. aureus.](image-url)
appropriate antibacterial effect at 99.96% against \textit{S. aureus} as shown in Figure 3f. Moreover, the AAP/EP composites also displayed similar increasing antibacterial activity trends and reached approximately antibacterial saturation including 5 wt% AAP at the sterilization ratio of 99.36%. However, the sterilization ratio of AAP/EP1 and AAP/EP3 against \textit{S. aureus} was about 35.28% and 82.28%, respectively, which were less efficient than AN/EP1 and AN/EP3 (Figure 3f). This may be that AAP is a natural \textit{A. annua} leaf powder, and the amount of the active bacterial ingredient is lower than artemisinin or AN.

Conversely, when the content was less than 5 wt% antibacterial agents, both AN/EP and AAP/EP composites revealed more efficient antibacterial performance against \textit{E. coli} than \textit{S. aureus}. The reason why it is easier to kill Gram-negative \textit{E. coli} than Gram-positive \textit{S. aureus} is that the membrane of Gram-positive bacteria is much thicker and more stable than that of Gram-negative bacteria.

Although the mechanism of AN sterilization has not been clearly understood, it can be assumed that such excellent antibacterial activity is related to terpenes and hydroxyls as shown in Figure 4a. Terpenes and hydroxyls may bind to DNA or specific proteins of bacteria so that their helical arrangement is disordered and the membrane permeability increases. Then, the biological processes of growth and propagation of bacteria are inhibited. Finally, the complexation between functional groups and bacteria make the cells inactive and die.

However, artemisinin also plays an important role in antibacterial process (33). As presented in Figure 4b, the peroxide bridge groups of artemisinin are likely dissociated by residual hydroxyl groups that may be induced to produce oxygen-free radicals. Thus, the radicals hinder the cell replication, leading to the deactivation of bacteria.

Since AAP contains both AN and artemisinin, it is certain that AAP has the potential in the antibacterial activity. In other words, the introduction of AAP into epoxy resins to improve antibacterial property is also a good choice. Therefore, 5% AN and AAP can be substituted for difficult-obtained artemisinin as addition in the epoxy matrix for further study.

### 3.2 Morphological property

As we all know, antibacterial performance is closely related to its morphology as well as dispersion.

![Figure 4](image-url) 

\textbf{Figure 4:} (a) Scheme of action of AN/EP in bacterial cells; (b) scheme of action of A/EP in bacterial cells.
The resulting fracture morphology of AN and AAP inclusion in the epoxy matrix can be characterized by scanning electron microscopy (SEM).

Figure 5 shows the morphology images of AN/EP5 and AAP/EP5. In Figure 5a, AN mixed with epoxy composites displayed very smooth surface without any disturbance. The interface between AN and epoxy matrix was very vague, and with larger magnification (Figure 5c), the phase separation of AN/EP composite can be hardly observed, which indicates that their compatibility is especially good. This is accordant with the good solubility during the preparation. Such excellent compatibility can be ascribed to the interaction between the terpene group of AN and the epoxide group of EP. Besides, their similarities in hydroxyl groups could be solvable easily in each other, resulting in a favorable compatibility.

Meanwhile, a homogeneous distribution of 5 wt% AAP in the epoxy matrix with rare agglomeration of the particles is shown in Figure 5b. Although with larger magnification, AAP particles (white dots in Figure 5d) in AAP/EP composite can still be observed, they were of nanometer size with uniform dispersion and blurring interface, indicating good interaction between AAP and EP. This phenomenon may be attributed to the absorption of energy by terpene and peroxide bridge groups, and such specific structure may prevent cracking and stress transfer.

The AN or AAP filler is uniformly dispersed in EP matrix, and no distinct defect can be observed. This indicates that the functionalized EP composite can effectively block the attack of bacteria. Owing to the good compatibility, the antibacterial activity of both composites is not restricted to the surface, and thus, they could be processed into not only films but also bulk shapes.

### 3.3 Mechanical property

For epoxy resin, not only its antibacterial property but also its mechanical performance plays a vital role in future application. Therefore, epoxy composites with different AN and AAP quantities were prepared, and their flexural properties were investigated as shown in Figure 6.

The flexural strength of the composites increased with the increasing AN and AAP content before containing 5 wt% content. The AN/EP5 composite showed a flexural strength of 68.17 MPa, which was 62.9% higher than that of pure epoxy. Such excellent improvement of AN/EP before 5 wt% addition may be attributed to the introduction of great-π-bonds and benzene rings from AN into flexible cycloaliphatic epoxy, leading to a substantial increase in rigidity.

![Figure 5: SEM images of (a) AN/EP5 5,000×, (b) AAP/EP5 5,000×, (c) AN/EP5 50,000×, and (d) AAP/EP5 50,000×.](image-url)
Besides, the good interaction between terpenes of AN and epoxide groups also benefits stress transfer. However, AN/EP7 showed a decrease in the flexural strength, which may result from some small molecules that act as plasticizers.

In contrast, AAP/EP maintained the original flexural strength due to a competitive system. On the one hand, AAP contains AN, which means the great-\(\pi\)-bonds from AN strengthen the interfacial action with EP, giving rise to an increase in the flexural strength. On the other hand, the insoluble solid grains in AAP leads to the heterogeneous phase that weakens the interaction between AAP and EP matrix, resulting in the reduction of AAP/EP in mechanical property. Therefore, the flexural strength of AAP/EP remained almost unchanged with the increasing amount of AAP. The results of the flexural strength were consistent with the observation from SEM.

Therefore, 5 wt% of AN with epoxy shows both good mechanical property and antibacterial activity, while 5 wt% of AAP with epoxy still maintains the flexural strength.

### 3.4 Thermal property

The thermal property is an important index of polymers. To study the effect of AN and AAP on the curing condition of epoxy resins, the curing temperature of pure epoxy, AN/EP5, and AAP/EP5 were investigated by DSC (Figure 7). The result showed that the curing temperature of pure epoxy was 150.3°C, AN/EP5 was 148.3°C, and AAP/EP5 was 148.4°C. When 5 wt% AN or AAP were added, the curing temperature of EP decreased. This means adding them into the epoxy matrix can accelerate the curing process of epoxy.

The glass transition temperature decides the application temperature range of polymer materials. Figure 8 shows the effect of AN and AAP on the glass transition temperature of epoxy resins. It is observed that the glass transition temperature of pure epoxy was 143.5°C, and there was a slight decrease in the glass transition temperature of epoxy containing 5 wt% of AN and AAP at 134.6°C and 143.4°C, but AN/EP5 and AAP/EP5 still remain in the glassy state under relative high temperature. The reason why the glass transition temperature of
AN/EP5 decreases is that the molecular weight of AN is lower as naphtha. Therefore, the small molecules inside may act as plasticizers decreasing its glass transition temperature. However, AAP is a solid powder smashed from A. annua and it contains both naphtha and other solid grains. Some fibers in solid grains (e.g., cellulose or collagen) may limit the movement of molecular chain, thus improving the glass transition temperature. Two opposite effects maintain the glass transition temperature of AAP/EP5. All in all, both AN/EP and AAP/EP composites are supposed to have good heat resistance.

4 Conclusion

Antibacterial epoxy resins containing AN and AAP from biological A. annua waste were prepared.

Both AN/EP and AAP/EP showed excellent antibacterial activity. Especially, EP with 5 wt% AN was approximately antibacterial saturation at the sterilization ratio of 100% and 99.96% against E. coli and S. aureus, respectively. Besides, AAP added into the epoxy composites as an up-and-comer also revealed the considerable antibacterial property at the sterilization ratio of 100% and 99.36% against E. coli and S. aureus, respectively. Besides, the addition of AN obviously increased the flexural strength of epoxy resins by 62.9%, while that of AAP could maintain the flexural strength. Furthermore, AN/EP and AAP/EP reflected stable thermal properties with almost unchanged curing temperature and glass transition temperature. Antibacterial AN/EP with the enhanced mechanical property has an enormous potential in biological and healthcare fields. Meanwhile, antibacterial AAP/EP would also be a less-cost choice in future sustainable development.

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