Endophytic fungi from medicinal plant *Garcinia cowa* Roxb. ex Choisy and their antibacterial activity

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**INTRODUCTION**

The evergreen *Garcinia cowa* plant is native to southwestern China but is also found in Asia, Bangladesh, Myanmar, Malaysia, Vietnam, Laos, and Cambodia. The fruit of this plant is known as cowa mangosteen or cowa fruit [1]. In West Sumatra, *G. cowa* Roxb is referred to as asam kandis. It is extensively dispersed across the Malay peninsula and Indonesia. The sour-tasting fruits are edible and are used as spices in Indonesia, particularly by the Minang tribes [2]. Young leaves of *G. cowa* are a popular choice as a vegetable, and the plant itself has a long history of use in folk medicine. The bark has been used as an antipyretic and antimicrobial agent [3]. On the other hand, the leaves and fruits are utilized to stimulate blood flow, alleviate indigestion and coughs, and promote laxative and expectorant effects [4]. Found in different parts of the plant, the chemical components known as xanthones and benzophenones have demonstrated anticancer [2], anti-inflammatory [5,6], antioxidant [7], antibacterial [8], and α-glucosidase inhibitory effects [9]. There have been reports that the peel extract of *G. cowa* fruit can inhibit the growth of certain bacteria, including *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus coagulan* [10] Methicillin-resistant *S. aureus* (MRSA), and *Salmonella typhimurium* [11].

Prior studies have demonstrated that endophytic microbes residing in plant tissue can synthesize secondary metabolite compounds found in plants. Secondary metabolite compounds produced by endophytic microbes can resemble those of their host plants. Venieraki (2017) reported that the secondary metabolite compounds produced by endophytic fungi...
are the same as the metabolites produced by their host plants. Diverse secondary metabolites will be made if the endophytic fungus is isolated and cultured under different conditions; however, these compounds will retain resemblances to those found in the host plant [12].

There is an insufficient number of research reports on endophytic fungi derived from the *G. cowa* plant, in contrast to the many studies on the bioactivity of secondary metabolic compounds from this plant. The growing issue of antibiotic-resistant bacteria and their control has necessitated the development of novel antibiotics for treating MRSA, which is now most urgent. Hence, this study was to isolate endophytic fungi from *G. cowa* plants and investigate their capacity to produce antimicrobial substances to impede the proliferation of pathogenic and drug-resistant bacteria, including *S. aureus*, MRSA, *E. coli*, and *C. albicans*.

**MATERIALS AND METHODS**

**Identification of sample material**

*Garcinia cowa* plants were taken from the Medicinal Plant Garden (KTO) of Andalas University, Padang, West Sumatra, during the rainy season in February 2023 (Fig. 1). The plant organs were stem bark, leaves, and roots with a sample weight of 10 grams each. The geographical coordinates of the garden are 0° 54' 36'' South Latitude and 100° 27' 45'' East Longitude. After successful identification, the specimen was stored at the Herbarium of Universitas Andalas in Padang, Indonesia. The voucher number was RIM002011 for plant authentication, and the letter number was 41/K-ID/ANDA/I/2023.

**Sample preparation**

For samples (leaves, stem bark, and plant roots) that are healthy, not infected with microbes, and have no insect bite wounds, surface sterilization is carried out and planted directly in the growth medium. The samples were washed with running water for 10 minutes and cut into four pieces with a length of approximately 1 cm each. The sample pieces underwent step-by-step sterilization involving immersion in 70% ethanol for 1 minute. They were put in a bleach solution with a length of approximately 1 cm each. The sample pieces underwent step-by-step sterilization involving immersion in 70% ethanol for 1 minute. They were put in a bleach solution (5.3% NaOCl) for 5 minutes and dipped again in 70% ethanol for 30 seconds. This sterilization process is carried out in laminar airflow. For leaf samples, scraping was carried out on the leaf surface. The sterilized pieces were placed on tissue paper and allowed to stand until the ethanol evaporated. Each part was put on Sabouraud dextrose agar (SDA) media with the cleavage surface attached to the agar medium. Incubation was carried out at 25°C (room temperature) for 3–5 days. On the 5th day, fungal growth was visible around the sample that had been placed on the agar medium. Endophytic fungi grown on SDA isolation media are gradually purified. Each endophytic fungal isolate that had grown was taken from the colonies on the surface of the media with a loop needle and transferred to another SDA medium for growth again. Each pure isolate was made in duplicate on agar slants. Each is a stock culture and culture for research [13].

**The cultivation of pure fungi isolates in rice medium and the preparation of extracts**

The pure isolate obtained at the purification stage was then cultured on rice media. Each fungus isolate was first cut into 1 × 1 cm slices in a Petri dish. It was then cultured in rice medium and incubated at room temperature for four to six weeks. The fungus isolate reaches its maximum growth potential when it covers the rice entirely. To produce fungus extract, optimally grown pure fungal isolates are macerated with EtOAc in a 1:1 ratio for twenty-four hours before rotary evaporation [13].

**Antimicrobial activity screening**

The test microbial suspension of *S. aureus* ATCC 25923, MRSA, *E. coli* ATCC 25922, and *C. albicans* (0.5 McFarland) was poured and spread evenly over the surface of the bacterial growth medium nutrient agar (NA), and the fungal growth medium SDA in a Petri dish. Sterile paper discs were dripped with endophytic fungal extract 5% in 10 µL dimethylsulfoxide (DMSO). As positive antibacterial and antifungal controls, we utilized chloramphenicol discs (Oxoid®) with a concentration of 30 µg/disc and nystatin discs (Oxoid®) with a concentration of 100 UI/disc. In addition, we utilized discs containing DMSO as negative controls. Then, each disk was arranged in an orderly on the prepared Petri dish. Petri dishes were incubated for 24 hours at 37°C for bacteria and 3–5 days at 25°C for fungi. Based on the clear zone that surrounded the paper disc that contained endophytic fungal extract, antimicrobial activity was observed and determined. A caliper is used to determine the diameter of the clear zone that is produced as a result [14].

**Preliminary phytochemical testing of extracts from endophytic fungi**

Secondary metabolite and phytochemical screening were performed to identify secondary metabolite groups from the EtOAc extract of endophytic fungi that showed antibacterial activity (inhibition zone more than 10 mm). A typical application of this qualitative chemical approach is determining whether terpenoids, alkaloids, phenols, flavonoids, or steroids are present. The terpenoid test was carried out using Liebermann–Burchard reagent, which changes the color.
of the extract to green, blue, or violet. The alkaloid test can be performed using the Dragendorff reagent, which causes the extract to turn orange. The phenolic test involves assessing the presence of phenolic compounds, and this can be done using various reagents, such as ferric chloride, which often produces a color change indicating the presence of phenols [15,16].

**Molecular identification**

Characterizing endophytic fungi entailed a macroscopic examination of each colony, during which its surface, coloration, and margin were evaluated. The molecular identification procedure entailed applying an Internal Transcribed Spacer (ITS) DNA barcode, which was constructed using primer pairs designed explicitly for fungi. The present study was carried out using the methodology that was delineated by Sandrawati et al. [17]. Large subunit ribosomal RNA, ITS 1, 5.8S ribosomal RNA, and ITS 2 are the four distinct components that comprise the ITS region. The sequencing procedure for the polymerase chain reaction product was carried out at First Base, a Malaysian laboratory facility. For species identification, the sequencing data were analyzed following the method described by Tallei and Kolondam [18] and then compared to the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree analysis was performed utilizing the neighbor-joining tree technique with 1,000 bootstrap replicates. The investigation was conducted using MEGA 7.0, a software application developed by Kumar et al. [19] which contains many sophisticated methods and tools for phylogenomics and phyomedicine. This major upgrade: MEGA has been optimized for use on 64-bit computing systems for analyzing bigger datasets. Researchers can now explore and analyze tens of thousands of sequences in MEGA. The new version also provides an advanced wizard for building timetrees and includes a new functionality to automatically predict gene duplication events in gene family trees. The 64-bit MEGA is made available in two interfaces: graphical and command line. The graphical user interface (GUI).

**RESULT AND DISCUSSION**

Medicinal plants remain to be utilized for their continued health benefits and have been used in traditional medicines for centuries. Presently, medicinal plants are being utilized to extract drugs derived from plants due to their high efficacy and minimal or absent adverse effects. However, because of the low rates at which these products build up in native medicinal plants, access to plant bioactive compounds is hampered. The natural resources of medicinal plants gradually run out [12]. Several studies have demonstrated that endophytic fungi present in medicinal plants can produce secondary metabolites that are pharmacologically active and comparable to those that their host plants produce. Since the identification of the endophytic fungus *Taxomyces andreanae* in 1993, which synthesizes the bioactive secondary metabolite taxol (paclitaxel) just like its host plant *Taxus brevifolia*, multiple subsequent investigations have definitively proven that endophytes are capable of producing plant-derived secondary metabolites [20,21]. The study’s findings suggest that endophytic fungi can be an alternative source for discovering new drugs. We have researched the bioactive compounds produced by endophytic fungi found in sea sponges, mangrove trees, and medicinal plants from West Sumatra, Indonesia. This research was based on the original premise [17,22–27].

A substantial amount of research has been carried out to determine the chemical composition and biological properties of various components of *G. cowa*. Previous studies have investigated the fresh leaves, fruits, and dried rinds of *G. cowa* and established that the principal constituents are organic acids and their lactones [28].

In the ongoing investigation of bioactive compounds generated by endophytic fungi residing in *G. cowa* plants, a total of thirteen strains of endophytic fungi were isolated from the leaves, stem bark, and roots of these plants (Fig. 2), which are recognized for their diverse bioactive oxygenated and prenylated xanthones [2].

To obtain endophytic fungal isolates, it was necessary to investigate the characteristics of the various colonies that were obtained from the leaves, stems, and roots of *G. cowa*. An equal number of endophyte isolates were found in the plant’s stems and leaves. Leaves of endophytic fungi are assigned the GCD code, stems are assigned the GCB code, and roots are given the GCA code. Five fungal strains were found in leaves, more than in other plant organs (Fig. 2), each isolated fungus was cultivated in rice media. The maximum growth, the secondary metabolite compounds produced by fungi are extracted with EtOAc. This solvent was selected because its characteristics make it semi-polar. This organic solvent attracted all nonpolar to semi-polar secondary metabolite components. The EtOAc extract was then tested for antimicrobial activity.

The results of the antimicrobial activity screening are shown in Table 1 and Figure 3. If the area around the disc shows a clear zone, the content of secondary metabolite compounds inhibits the growth of bacteria and fungi. Out of the endophytic fungi collected, 80% could impede the growth of *S. aureus*, MRSA, and *E. coli* in the experiment. However, none of these fungi showed any activity against *C. albicans*.

At a concentration of 5%, four fungal extracts were observed to be effective against *S. aureus* and MRSA, while five fungal extracts were used to combat *E. coli*. Of all the fungi extracts, the fungal isolate known as GCA3 had the highest inhibition diameter, measuring more than 10 mm. The inhibition zone measurements for *S. aureus*, MRSA, and *E. coli* were 15.05 ± 0.51 mm, 13.48 ± 0.15 mm, and 14.68 ± 0.5 mm, respectively (Table 2). In addition, the extracts of GCA3 were subjected to phytochemical screening to determine the presence of bioactive compounds, specifically those with strong antimicrobial properties. The presence of phenolic and flavonoid compounds in the GCA3 extract was confirmed by phytochemical screening (Table 3). In addition, it has been demonstrated that the extract of the *G. cowa* plant contains secondary metabolites. These secondary metabolites include phenolics, triterpenoids, flavonoids, and xanthones within the extract.

The GCA3 fungus has a colony structure that looks like hard skin. The front of the colony is bluish–blue, and the back is yellow to orange. The walls of conidiophores are...
smooth. Fialid is in the shape of a bottle. The conidia grow in columns that are round to nearly round. The walls are soft, the outside is a little rough, and they are greenish (Fig. 4).

In sequence, molecular identification showed that GCA3 was 100% identical to *Penicillium citrinum*. The neighbor-joining method with a bootstrap value of 1.000 was used to limit the phylogenetic tree (Fig. 5). The endophytic fungus *P. citrinum* is known to make several bioactive compounds with different biological effects. Tanzawaic acid is a polyketide compound initially obtained from *P. citrinum* in Japan in 1997. This compound exhibits antimicrobial activity against a range of bacteria, including *S. aureus*, *Salmonella* sp., *Klebsiella pneumoniae*, *E. coli*, *B. cereus*, *Proteus mirabilis*, *Enterococcus faecalis*, and *C. albicans* [29]. Penicitrinine A derived from *P. citrinum* exhibits anti-proliferative properties on various tumor cells, including HGC-27, SPC-A1, and A-375. Furthermore, this fungus also generates secondary metabolites that, by downregulating Bel-2 expression and stimulating Bax and SPC-A1 cells [30], can cause apoptosis in A-375 cancer cells. According to Khamthong et al. (2012), the *P. citrinum* PSU-F51 exhibits bioactivity in the form of cytotoxic and antibacterial effects on KB cells [31]one isochroman (3. Penicitrinine A). According to El-Neketi et al. (2013), cristiquinocromexhibits cytotoxic activity on L5178Y lymphoma cells [32]. Kumar et al. (2021) published findings on extracting the endophytic fungus *P. citrinum* from *Azadirachta indica*. The secondary metabolites these fungi produce exhibit substantial antimicrobial activity against bacteria and fungi harmful to humans. The area where bacterial growth was inhibited ranged from 15 to 20 mm for *S. aureus*, *E. faecalis*, and *Aeromonas*

![Figure 2. The pictures of isolated fungal endophytes from *Garcinia cowa* Roxb. ex Choisy growing on SDA.](image)

![Figure 3. The agar plate pictures of the inhibition zone of fungal isolate extract against the growth of *S. aureus* (A), MRSA (B), and *E. coli* (C).](image)

### Table 1. Antimicrobial activity of endophytic fungi from *Garcinia cowa* Roxb. ex Choisy growing on SDA.

<table>
<thead>
<tr>
<th>Fungal code</th>
<th>Organ</th>
<th>Average of Inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>GCA1</td>
<td>Root</td>
<td>11.23</td>
</tr>
<tr>
<td>GCA2</td>
<td>Root</td>
<td>11.9</td>
</tr>
<tr>
<td>GCA3</td>
<td>Root</td>
<td>14.11</td>
</tr>
<tr>
<td>GCB1</td>
<td>Stem</td>
<td>10.37</td>
</tr>
<tr>
<td>GCB2</td>
<td>Stem</td>
<td>9.91</td>
</tr>
<tr>
<td>GCB3</td>
<td>Stem</td>
<td>7.42</td>
</tr>
<tr>
<td>GCB4</td>
<td>Stem</td>
<td>11.60</td>
</tr>
<tr>
<td>GCD1</td>
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<td>-</td>
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<td>11.43</td>
</tr>
<tr>
<td>GCD4</td>
<td>Leaf</td>
<td>-</td>
</tr>
<tr>
<td>GCD5</td>
<td>Leaf</td>
<td>8.09</td>
</tr>
<tr>
<td>GCD6</td>
<td>Leaf</td>
<td>8.55</td>
</tr>
</tbody>
</table>
The highest level of inhibition, measuring 29 mm, was observed against *Trichophyton mentagrophytes*. Through the utilization of thin layer chromatography, gas chromatography–mass spectrometry, proton nuclear magnetic resonance (H NMR), and Carbon-13 (C13) nuclear magnetic resonance (13C NMR) techniques for characterization and structure elucidation, it has been determined that this bioactive compound is identical to milbemycin [33].

As a unique microorganism, *P. citrinum* can produce bioactive compounds identical or similar to those from its host plant and other bioactive components. The investigation’s findings into the endophytic fungi found on *G. cowa* plants shed light on the interaction between these microorganisms and their host plants and the variety of naturally occurring bioactive substances these fungi produce. The findings of this research will impact the productivity of several potential candidate compounds by using genetic engineering, microbial fermentation projects, and other effective techniques that can be developed well.

### Conclusion

The medicinal plant *G. cowa* offers extensive biological resources in the form of bioactive compounds with significant antibiotic, antioxidant, and anticancer agent potential. This study isolated thirteen endophytic fungi from these plants’ roots, stems, and leaves. The fungal strain GCA3 exhibits significant antibacterial activity, effectively inhibiting the growth of pathogenic bacteria such as *S. aureus* and MRSA. GCA3 is the same as *P. citrinum*. A significant obstacle is presented by isolating bioactive chemicals from this fungus to conduct additional research and develop new antibiotic candidates.

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### Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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### Conflicts of Interest

The writers have identified no conflicts of interest.
ETHICAL APPROVALS
This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY
All of the collected and analyzed data is in this research article.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY
The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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