Isolation of active compounds as anti-acne from ethanolic extract of Artocarpus camansi leaves

AZALIA IZDHIHAR AZWAR¹,², MUHAMMAD BAHI²*, ROSNANI NASUTION², NURDIN NURDIN², MARIANNE MARIANNE³

¹Master of Science, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
²Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia.
³Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155 Indonesia

Abstract. Acne vulgaris or acne is a chronic inflammatory skin disease in adolescents and adults affecting one self-confidence. The result of the research shows the ethanol extract, subfraction, pure isolate, and gel extract of Artocarpus camansi are active in inhibiting the growth of Propionibacterium acnes. The ethanol extract with the highest inhibition zone was at the concentration of 0.5% and 2% with 9.1±0.62 mm and 8.5±0.7 mm with the activity of 47.07% and 43% respectively (in comparison with the chloramphenicol 30 μg 19.33±0.47mm). The gel extract had the highest activity at 2%, with an inhibition zone of 8±0.50 mm and the activity of 43.97%. Anti-acne activity for all subfractions had an activity of 32.5-33.3% while the pure isolate was at 42.10 - 45.63%. Physical properties of the gel extract were evaluated for four weeks by examining the pH test obtained with a range of 5.8-6.7, viscosity in the range of 18460-19668 cP, spreadability in the range of 5.45-7.20 cm, adhesion observation above 4 seconds, and homogeneity in a concentration of 0.5-7.5%. The pure isolate has a melting point of 147 °C, presumed to be γ-sitosterol and appropriate with the screening phytochemicals results with the presence of steroid.

Keywords: Artocarpus camansi, Anti-acne, Gel, Medicinal plant, Propionibacterium acnes

INTRODUCTION

Acne vulgaris is a common chronic skin disease in the pilosebaceous unit that often affects adolescents [1] Propionibacterium acnes (P. acnes) is one of the causes involved in acne inflammation [2]. Although not life-threatening, this skin disease can affect the quality of life and produce social and economic impacts on the sufferer. It is necessary to search for appropriate and effective drugs to tackle the issue. Artocarpus camansi (A. camansi) plant is one of the plants Family Moraceae well-known to the public as a source of medicine. Scientific research has been carried out on the leaves, bark, roots, and fruit, showing that they are helpful in reduce concentartions of blood sugar [3-8]. Artocarpus camansi is also a good antioxidant and antibacterial activity against Staphylococcus aureus with a minimum inhibition concentration (MIC) of 25 mg/mL, 25 mg/mL for Escherichia coli, and 50 mg/mL for Pseudomonas aeruginosa [9]. Flavonoids in A. camansi are also known to have effects such as anti-inflammatory, antibacterial, antiviral [10], and antioxidants [11]. However, research on A. camansi against bacteria causing acne, namely the bacterium P. acnes, is still lacking, even though other species from the Moraceae family are active in inhibiting the growth of other acne-causing bacteria. Various oral and topical anti-acne preparations are on the market, but gel forms are preferred. Gel forms are often better for acne treatment. Because the gel has cooling, moisturizing, and easy to use [12]. Further, gel preparation could be quickly absorbed, so it is more effective to help absorb active ingredients in the infected area [13].

METHODOLOGY

Materials

Leaves of Artocarpus camansi were collected from Bireuen, Aceh, Indonesia, in December 2020 and were identified in the Department of Biology of Universitas Syiah Kuala, Indonesia. Kingdom: Plantae, Sub Kingdom: Tracheobionta, Super Division: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Sub Class: Hamamelididae, Order: Urticales, Family: Moraceae, Genus: Artocarpus JR Frost & G. I, Species: Artocarpus camansi Blanco. The bioindicador used in this study was the bacterium Propionibacterium acnes, Strain (ATCC 27853) from the Department of Biology, Universitas Sumatra Utara (USU).
The chemical solvents used are n-hexane, ethyl acetate, and ethanol. NaCl 0.85%, 70% alcohol, lactose broth (Oxoid), Media Mueller-Hinton Agar (MHA) (Oxoid), Thin Layer Chromatography F254 (Merck) and silica gel 60 GF254 (Merck) and gel-making materials: Carbolip 940P, glycerin, triethanolamine were purchased at Rudang Jaya, Indonesia. Blank disk (Oxoid), antibiotic chloramphenicol disc 30µg (Oxoid) were purchased at Mitra Sejahtera Laboratorium, Indonesia.

GC-MS QP 2010 Ultra (Shimadzu, Japan) Electric balance (Mettler Toledo, Japan), Rotary evaporator (Buchi R-300), pH meter 710 A Thermo Electron Orion, Thermo Scientific Haake viscometer, scatter power test equipment, and glassware.

Extractions of A. camansi leaves
A 425 g of dried samples of A. camansi leaves cleaned from dirt and air-dried at room temperature. Thereafter were cut into small pieces and macerated using n-hexane solvent for 3 x 24 h. The filtrate was filtered; the residue was dried and then macerated again using ethanol for 3 x 24 h. The filtrate of ethanol was evaporated using a vacuum rotary evaporator and produced 34.01 g ethanol extract (8% yield of extract). The ethanol extract was used for phytochemical analysis [14], anti-acne activity assay [15] and evaluated physical properties gel extract. The ethanol extract was characterized using GC-MS and separated by gravitational column chromatography to obtain pure compounds.

Phytochemical analysis
The ethanol extract was used for phytochemical analysis such as alkaloids, terpenoids, steroids, saponins, flavonoids and phenolic [14].

Alkaloids identifications
Ethanol extract of A. camansi leaves was added with 0.05 N of ammonia-chloroform solutions. The liquid phase was collected into a test tube. Then, 5 mL of HCl 0.5 N was added and shaken. The mixture would be two layers. The top layer was a solution in HCL, and the bottom layer was a solution in chloroform. The solution in HCl was tested with three reagents. The formation of a yellow precipitate indicated the test with Mayer’s reagent. The formation of a brown precipitate indicated the test with Wagner’s reagent. Additionally, the formation of an orange or red precipitate indicated the test with Dragendorff’s reagent [14].

Terpenoids and steroids identifications
Ethanol extract of A. camansi leaves was added with a few drops of Liebermann- Burchard. The color then changed into red, which indicated terpenoids, while green or blue color indicated steroids [14].

Saponins identifications
Ethanol extract of A. camansi leaves was added with the aquadest and shaken in a test tube for 5 min. A foam forming about 5 min which indicated saponins [14].

Flavonoids identifications
Ethanol extract of A. camansi leaves was added with 0.5 g of magnesium powder and HCl 0.5M. If the color changes into pink or purple, it indicated flavonoids [14].

 Phenols identifications
Ethanol extract of A. camansi leaves was added with FeCl3. The color then changed into a deep green which indicated phenolics compounds [14].

Preparation of anti-acne gel
Composition of ethanol gel extract of A. camansi leaves was carried out based on (Table 1) in previous research conducted by Sharma [16] and then were modified.

Carbolip was developed with the help of water stirred gradually. Then, added triethanolamine and glycerin. The mixture were stirred constantly until homogeneous, next aquadest were added up to 100 mL while stirring gel base was formed. After that, the extract at various concentrations were added a stirred again until homogeneous [16].

Anti-acne activity assay
The anti-acne activity was assayed using the disk diffusion (Kirby-Bauer) method, with an incubation period of 24 hours, and three times of repetitions. As many as 30 mL of Mueller-Hinton Agar (MHA) medium were prepared and solidified onto a petri dish. The ethanol extracts of A. camansi leaves were made in various concentrations, namely 0.5; 2; 5; 7.5; and 12% (w/v).

Pure bacterial colonies of P. acnes in Nutrient Borth medium were taken with a micropipette, they were suspended in a sterile nutrient broth medium for regeneration then incubated and shaken at 150 rpm for 24 h. Right after, microorganisms were moved and suspended again in 5 mL of 0.9% NaCl solution to

Table 1. Composition of gel material (%)

<table>
<thead>
<tr>
<th>Materials</th>
<th>Composition of gel material (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>-</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td>0.5</td>
</tr>
<tr>
<td>Carbopol</td>
<td>0.5</td>
</tr>
<tr>
<td>Glycerin</td>
<td>2.0</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.1</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.2</td>
</tr>
<tr>
<td>Aquades</td>
<td>96.9</td>
</tr>
</tbody>
</table>

Note: the composition of the ingredients were all made in the v/v, except for ethanol extract of A. camansi leaves that were using the w/v composition

TEA = Triethanolamine
reach the turbidity standard of 0.5 by McFarland or equal to 1.5x10^8 cfu mL^-1 [17]. Then they were swabbed into the media. Antibiotic chloramphenicol 30 μg and ethanol solvent were used as positive and negative control respectively.

**Evaluation gel extract**

Evaluation of the gel extract was carried out by observing its pH [18], spreadbility [19], viscosity [20], adhesion [21], and homogeneity [22] for four weeks with three repetitions.

**Determination of pH**

2 g of ethanol gel extract was put into a test tube to measure the pH meter. The pH of good gel preparation is the range of 4.5-8 [18].

**Determination of spreadbility**

0.5 g of ethanol gel extract was placed between round glass. Then a load of 100 g was placed on the glass and left for 60 sec to measure the constant diameter [19].

**Determination of viscosity**

1 g of ethanol gel extract was put into a test tube to evaluate the viscosity. The process was conducted on a Thermo Scientific Haake viscometer [20].

**Determination of adhesion**

0.5 g of ethanol extract gel was placed between round glass. They were pressured with a load of 50 g for 5 min. Further, the object in the glass is mounted on the test equipment that is giving a load weighing 500 g. The time required for the two glass separate was noted [21].

**Determination of homogeneity**

0.5 g of ethanol gel extract was placed between round glass. The extract was declared homogeneous if no coarse details were visible [22].

### Table 2. Phytochemical test results of ethanol extract of *A. camansi* leaves

<table>
<thead>
<tr>
<th>Phytochemical contents</th>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Drangendorf</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>TLC with</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Vanilin sulfur reagen</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Liebermann-Burchard</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.5 g Mg dan hcl</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Water and</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Shaking</td>
<td></td>
</tr>
<tr>
<td>Phenolic</td>
<td>Fecl</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) presence of compounds (-) absence of compounds

**RESULTS AND DISCUSSION**

**Phytochemical contents**

The result of phytochemical contents of ethanol extract of *A. camansi* leaves is shown in Table 2. Soegandi and Amelia [10] reported that the ethanol extract of *A. camansi* leaves contains secondary metabolites of alkaloids, terpenoids, tannins, phenolics, flavonoids, saponins, and steroids.

**Anti-acne activity**

Anti-acne activities from ethanol extract and gel extract were presented in Table 3. The result showed that the concentrations of 0.5 and 2% were the optimum concentrations for inhibiting the growth of *P. acnes* bacteria. They had an inhibition zone of 9.10±0.62 and 8.50±0.70 mm, with the percent activity of 47.07 and 43.97%, compared to the positive control of chloramphenicol 30 μg with inhibition zone 19.33±0.47 mm. This inhibition zone was categorized as the moderate. The classifications of inhibition zone were in four categories; >20 mm, very strong; 10-20 mm, strong; 5-10 mm, medium; and <5 mm, no response [23].

Ethanol gel extract of *A. camansi* leaves had an inhibition zone more minor than the inhibition zone of each extract. The best gel inhibition zone was at a concentration of 2% extract with a diameter of 8.5 mm, which was considered as medium category [23]. Further, the percentage of activity was 47.07%. The difference in the concentration of the extract produced different inhibition zones for the gel. Percentage of activity was calculated by the following equation:

\[
\text{Activity power} \% = \frac{\text{inhibition zone of extract} \times 100}{\text{inhibition zone of control positive}}
\]

Note * Inhibition zone of extract/sub fraction/pure isolate/gel extract.

### Table 3. Anti-acne activities of the ethanol extract and gel of *A. camansi* leaves

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of inhibition* (mm)</th>
<th>% Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Extract</td>
<td>8.30±0.471</td>
<td>42.93%</td>
</tr>
<tr>
<td>Extract 0.5 %</td>
<td>9.10±0.623</td>
<td>47.07%</td>
</tr>
<tr>
<td>Extract 2%</td>
<td>8.50±0.707</td>
<td>43.97%</td>
</tr>
<tr>
<td>Extract 5%</td>
<td>7.30±1.247</td>
<td>37.76%</td>
</tr>
<tr>
<td>Extract 7.5%</td>
<td>8±10.414</td>
<td>41.38%</td>
</tr>
<tr>
<td>Extract 12%</td>
<td>7±10.374</td>
<td>37.76%</td>
</tr>
<tr>
<td>Gel Extract 0.5 %</td>
<td>6.33±0.471</td>
<td>32.74%</td>
</tr>
<tr>
<td>Gel Extract 2%</td>
<td>8.50±0.50</td>
<td>43.97%</td>
</tr>
<tr>
<td>Gel Extract 5%</td>
<td>7.00±0.816</td>
<td>36.21%</td>
</tr>
<tr>
<td>Gel Extract 7.5%</td>
<td>7.00±0.816</td>
<td>36.21%</td>
</tr>
<tr>
<td>Gel Extract 12%</td>
<td>7.00±0.816</td>
<td>36.21%</td>
</tr>
<tr>
<td>Positive control (+)</td>
<td>19.33 ± 0.471</td>
<td>-</td>
</tr>
<tr>
<td>Negative control (-)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: *Values are expressed as mean ± SD were calculated from three repetition.
Isolation of active compound as anti-acne from ethanol extract Artocarpus camansi leaves
(Azalia Izdihiar Azwar, Muhammad Bahi, Rosnani Nasution, Nurdin, Marianne)

A comparison of anti-acne activities of ethanol extract and gel extract can be seen in Figure 1. The data showed that the ethanol extract of A. camansi leaves, after formulated into a gel, experienced a decrease in anti-acne activity. This situation occurred because active compounds were trapped in the gel extract, which affected the active substance's diffusion process in the media [24]. Gel at a concentration of 2% produced a good consistency, not too dense and not too liquid. Presumably, it was able to release the active substance from the ethanol extract of A. camansi leaves into the test medium properly.

Several factors affected the size of the inhibition zone on bacteria, like plant sensitivity, reactions with the active ingredients, incubation medium, temperature [25], environmental pH, media components [26], colony density, metabolic activity of microorganisms, and impurities [27]. The results of research conducted by Vianey et al (2018) stated that the ethanol extract from the leaves of A. camansi had antibacterial activity against S. aureus and E. coli bacteria with inhibition zones of 11 and 10 mm. Soegandi and Amelia (2020) [10] uncovered that there were antibacterial activities of the ethanol extract of A. camansi leaves against S. dysenteriae and B. subtilis bacteria with a MIC of 6.25%.

The positive control used in the anti-acne test was 30 µg chloramphenicol antibiotic disc. Chloramphenicol is an antibiotic with an action mechanism that inhibits the bacterial protein synthesis process [28]. The negative control did not produce any inhibition zone because organic solvents did not affect the bioactivity of secondary metabolites against pathogenic bacterial species [29]. Anti-acne activities resulting in this conclusion were due to the content of secondary metabolites such as, steroids [30], flavonoids [31], alkaloids [32], terpenoids [33], tannins, [34], phenolics [35].

**Isolation of ethanol extract of A. camansi leaves and anti-acne pure isolate**

Based on rechromatography, crude extract resulted in 62 fractions, later categorized into three subfractions. They were tested for their anti-acne activities (Table 4).

All subfractions had the relative same activities, so this subfraction was collected and further separated by gravity column chromatography to obtain pure compounds. The results of purity testing with three eluent systems, i.e., chloroform (100%); n-hexane:ethyl acetate (50:50); and (70:30). The Rf values of pure isolates obtained were 0.68, 0.5, and 0.72, respectively. The pure compound melted at 147°C. Based on the existing literature, it was suspected that the pure compound obtained was γ-sitosterol, which had a melting point range of 147-148°C [36].

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of Inhibition* (mm)</th>
<th>% Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subfraction I</td>
<td>6.50± 0.05</td>
<td>32.50%</td>
</tr>
<tr>
<td>Subfraction II</td>
<td>6.66± 0.00</td>
<td>33.30%</td>
</tr>
<tr>
<td>Subfraction III</td>
<td>6.66± 0.00</td>
<td>33.30%</td>
</tr>
<tr>
<td>Positive control (+)</td>
<td>20± 0.00</td>
<td>-</td>
</tr>
<tr>
<td>Negative control (-)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: All of subfraction were tested in 12% w/v
Note: *Values are expressed as mean ± SD were calculated from three repetition

**Figure 1.** Comparison of inhibition zones of extract and gel extract.

**Table 4.** Anti-acne of sub fraction ethanol extract of A. camansi leaves

**Figure 2.** The spectrum of mass spectrometry was suspected γ-sitosterol.
Isolation of active compound as anti-acne from ethanol extract *Artocarpus camansi* leaves
(Azalia Izdihar Azwar, Muhammad Bahi, Rosnani Nasution, Nurdin, Marianne)

Based on the results of GCMS fragmentation pattern (Figure 2), it resulted the peaks of m/z as follows: 414, 396, 381, 354, 329, 303, 273, 255, 231, 213, 199, 185, 173, 159, 145, 133, 119, 107, 95, 81, 57, 43, and 41. This peak showed the similarity to the peak of the γ-

**Figure 3.** The fragmentation pathways of the γ-sitosterol compounds [37].

**Figure 4.** Inhibition zone pure isolate against *P. acnes*.

**Table 5.** Inhibition zones of pure isolate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of Inhibition* (mm)</th>
<th>% Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure isolate (0.5%)</td>
<td>8,67 ± 0,47</td>
<td>45,63</td>
</tr>
<tr>
<td>Pure isolate (7.5%)</td>
<td>8,00 ± 0,00</td>
<td>42,10</td>
</tr>
<tr>
<td>Positive control (+)</td>
<td>19,00±0,81</td>
<td>-</td>
</tr>
<tr>
<td>Negative control (-)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: *Values are expressed as mean ± SD were calculated from three repetition

**Figure 5.** Graphic data for (a) pH, (b) viscosity, (c) spreadability, and (d) adhesion.
sitosterol compounds. The fragmentation pathways of the γ-sitosterol compounds were presented in Figure 3.

Anti-acne activity of pure isolate can be seen in Table 5 and Figure 4. The result of the anti-acne pure isolate was 42.10% and 45.63%, which was not much different from the activity of the crude extract. The most active concentration was 0.5%. That means the compounds that acted as anti-acne also played a significant role in the extract.

**Evaluations of ethanol gel extract**

Evaluations of gel extract with various concentrations had been evaluated for four weeks testing the pH, viscosity, spreadability, and adhesion (all presented in Figure 5).

The pH test on the ethanol extract of *A. camansi* leaves could be identified by the level of acidity. Good levels of pH acidity ranged from 4.5-8 [18]. Based on the data, the pH measurements ranged from 5.85 to 6.76. Meaning that ethanol gel extract was safe for use on the skin. Increasing the concentration of ethanol extract of *A. camansi* leaves in the gel caused the decreasing of the pH value (becoming more acidic). Further, the storage factor of pH value became more acidic than the previous pH. Presumably, the storage period of the gel extract evolved to be more concentrated so that the physical properties related to pH changed. Based on viscosity data in the observation, ethanol gel extract ranged from 18460 to 19668 cP. Good topical gel preparation must align with SNI 16 4399-1996 with the viscosity value of 6000-50000 cP [38]. The viscosity value of the gel extract decreased along with the increased concentration of ethanol extract. Based on Figure 3 (b), storage time affected the viscosity. This statement was supported by Ida and Noer, 2012 [39]. One of the causes was syneresis, which was the release of the liquid trapped in the gel to come out to the surface [40]. Moreover, it was suspected that this occurred due to the influence of temperature and storage method of the gel preparation.

Adhesion for topical preparations was not too strong, causing skin breathing to be obstructed and it was not too weak to reduce the therapeutic effect [41]. Good topical preparation had an adhesion value of not less than 4 seconds [42]. In all concentrations, the ethanol gel extract of *A. camansi* leaves had good adhesion, above 4 seconds. Based on data, storage time made adhesion increased because the solvent used in gel extract was evaporated.

Ethanol gel extract was best homogeneity with concentrations of 0.5; 2; 5; and 7.5%. Gel extract at the concentration of 12% was not as good as gel preparation. This is presumably due to the addition of mass extract of concentration of 12% inhomogeneous.

**CONCLUSION**

It can be concluded that the extracts, pure isolates, and gel extracts from *A. camansi* leaves demonstrated relatively moderate inhibition power against *P. acnes*. The gel extract had an inhibition zone with a concentration of 2% and inhibition power of 43.97%, compared to the positive control of chloramphenicol 19.33mm. The pure compound had a melting point of 147-148 °C which is suggested to be γ-sitosterol.

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