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A Comparative Study of Total Tannin Contents and Antimicrobial Activities in Methanol Extracts of Rhizophoraceae Species

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Abstract

Rhizophoraceae is a large true mangrove family that produces mangrove tannins. Tannins have the potential to be used as antimutagenic, anticancer, antimicrobial, and antioxidant agents in the pharmaceutical and medical fields. The aim of the research was to determine the total tannin content, antimicrobial activity, and the correlation between the two in methanol extracts of four plant parts (roots, bark, leaves, and fruit/hypocotyl) from five Rhizophoraceae species, namely *Bruguiera cylindrica*, *Bruguiera gymnorrhiza*, *Ceriops decandra*, *Rhizophora apiculata*, and *Rhizophora mucronata* originating from the Langsa mangrove forest, Aceh, Indonesia. Folin Ciocalteu colorimetric method and tannic acid as a standard were used for the total tannin content (TTC) test. The disc diffusion method was used to conduct antimicrobial tests against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans* bacteria at a 50% extract concentration. TTC content in Rhizophoraceae ranged from 8.17 ± 1.36 mg TAE/g to 813.35 ± 18.72 mg TAE/g ($p < 0.05$). The highest levels were found in *C. decandra* roots (813.35 ±18.72 mg TAE/g), *C. decandra* bark (704.36 ± 12.11 mg TAE/g), and *R. apiculata* bark (651.23 ± 2.36 mg TAE/g). The extract has moderate to strong antibacterial activity against gram-positive bacteria *S. aureus*, with an inhibition zone range of 6.64 ± 0.80 mm -15.02 ± 0.32 mm. The highest antibacterial activity was observed in the inhibition zones for *S. aureus* bacteria, with *C. decandra* fruit extract (15.02 ± 0.32 mm), *R. apiculata* bark (14.78 ± 0.90 mm), and *C. decandra* leaves (14.44 ± 1.18 mm) exhibiting the highest levels. TTC and antibacterial activity of *S. aureus* in Rhizophoraceae extracts showed a moderate correlation (Pearson correlation coefficient $r = 0.566$, $p < 0.05$). According to the results, it was concluded that Rhizophoraceae has the potential to produce optimal tannins that can be used as antibacterial agents against *S. aureus*.

1. Introduction

The Rhizophoraceae family stands as a prominent representative of true mangroves, boasting the highest number of members among all such mangrove families. In Indonesia, Rhizophoraceae encompasses four genera and a total of eleven tree species [1]. Some of the species found in Indonesia include *Bruguiera cylindrica*, *B. gymnorrhiza*, *B. parviflora*, *B. Sexangula*, *Ceriops decandra*, *C. tagal*, *Kandelia candel*, *Rhizophora apiculata*, *R. lamarckii*, *R. mucronata*, and *R. stylosa* [2].

Rhizophoraceae are commonly used in traditional medicine in China, America, India, Bangladesh, Burma, Thailand, Peninsular Malaysia, and Indonesia [3]. *B. gymnorrhiza* species, for example, are used to treat herpes, diarrhea, and intestinal worms [4, 5]. Indians, the bark and roots of *B. cylindrica* are used to treat diabetes and viral fever [6]. A decoction of *C. decandra* leaves and bark is used to treat bleeding and ulcers, and fresh leaf juice is given to mothers during postpartum [7, 8]. Elephantiasis, amoebiasis, and dysentery are all treated with *R. apiculata* plant parts [9, 10]. Furthermore, in China, the bark of *R. mucronata* is used to treat leprosy and diarrhea, and in Japan, the leaves are used as an antiseptic and toner [11].

The need to harness the pharmacological potential of natural plants has gained significant attention in recent years [12–15]. Several previous studies on the pharmacological properties of Rhizophoraceae, including antimicrobial properties, have been thoroughly examined [16, 17], anticancer [7, 16, 18, 19], anti-diabetic [20, 21], hepatoprotective [4, 22, 23], anti-hypertension [24], antioxidants [25] and anti-inflammatory [26]. Rhizophoraceae, a mangrove species, has a diverse chemical composition that includes terpenoids (16.25%), tannins (12.5%), steroids (10.0%), alkaloids (9.38%), saponins (8.75%), flavonoids (8.75%), and glycosides (8.13%) [3]. According to Rafathi et al., the most common chemical constituents found in Rhizophoraceae are terpenoids and tannins [27]. Rhizophoraceae is widely used in the charcoal industry in Malaysia, and its bark is capable of producing high tannins [28]. This mangrove species contains up to 70% tannin, which is responsible for astringent, anti-diabetic, anti-rheumatic, and hypotensive properties [3]. In another study, tannin isolated from the roots of *B. gymnorrhiza* was found to be responsible for its antinociceptive and antidiarrheal properties [29]. Phenolics and flavonoids are important phytoconstituents in this plant that contribute to its pharmacological properties. [25]. However, very few people have reported on the phytochemical content and pharmacological activity of tannins, which act as Rhizophoraceae phytoconstituents.

Tannins are secondary metabolites found in many plant parts that help plants defend against fungi, bacteria, and insects, as well as survive during drought [30]. Tannin's potential applications include the leather processing industry, the wood industry, metal anti-rust, water treatment plants, animal food, and the production of advanced materials [31, 32]. Notably, tannins also hold promise for utilization in the pharmaceutical and medical sectors. They exhibit pharmacological properties, such as antimutagenic, anticancer, antimicrobial, and antioxidant effects [30].

The tannin content of mangrove plants is still being studied because this type of mangrove contributes to the world's thickest tannins [31]. In comparison, Long-term research on wattle tannin from the bark of the *Acacia mearnsii* tree, including in vivo activity tests and clinical trials, has now resulted in health food supplement products in Japan that can last up to ten years without side effects [33, 34]. Other studies have discovered that Rhizophoraceae extract has a high tannin content as well as antimicrobial activity [35–37]. Several studies also found that the antioxidant activity of Rhizophoraceae increased as tannin concentration increased [38–40]. However, there is currently no comprehensive information on the total tannin content of mangroves in the large family Rhizophoraceae.

The current research conducted was an initial screening aimed at assessing both the tannin content and antimicrobial activity within the Rhizophoraceae species. If tannins from Rhizophoraceae extracts are shown to inhibit the growth of pathogenic bacteria and fungi in vivo, the extract will be studied further to determine the optimal concentrations for controlling the human gut and skin microbiome. As a result, in the future, the extract could be used to make tea, food supplements, or topical medicines that are beneficial to human health.

The aim of the research was to determine the total tannin content and antimicrobial activity of methanol extracts of four plant organs (roots, bark, leaves, and fruit/hypocotyl) from five Rhizophoraceae species, namely *B. cylindrica, B. gymnorrhiza, C. decandra, R. apiculata*, and *R. mucronata*. Subsequently, determine whether there is a correlation between tannin content and antimicrobial activity. In an era when most researchers screen thousands of plants for new compounds, Rhizophoraceae tannin content and antimicrobial activity were screened. This is critical so that we can determine the quantity and quality of phytochemicals based on their bioactivity in plants and use them as potential candidates for the development of pharmaceutical drugs.

2. Materials and Methods

2.1. Sample preparation of Rhizophoraceae

Research samples consisted of roots, bark, leaves, and fruit from five species of Rhizophoraceae (*B. cylindrica, B. gymnorrhiza, C. decandra, R. apiculata,* and *R. mucronata*) collected from the Langsa Mangrove Forest Area, Aceh, Indonesia. The diameters of the sampled tree trunks ranged from 10 to 30 cm. Before being cut into small pieces, the samples were cleaned with running water. Samples were dried in the shade for 20 days until the plant weight did not change. The samples were placed in an airtight container for further processing. Plant identification was performed at Syiah Kuala University's Department of Biology in Banda Aceh, Indonesia.

2.2. Plant Extraction

A total of 20 dry samples weighing 100 g were weighed. Each sample was soaked for 24 hours in 1000 mL of methanol at room temperature (25 °C). The extract is filtered using a glass funnel and Whatman filter paper No. 1. Each filtrate was concentrated until thick using a rotating Rotary evaporator (Büchi Labortechnik, Germany) at low pressure and controlled temperature (40-50 °C). Next, it is dried over a water bath at 40 °C and left at room temperature (25 °C) until completely dry. The dry extract is then stored at room temperature in an airtight vial before further use. Maceration methods were carried out based on previous research [41]. For each extracted sample, the following code is used: Methanol extract samples of *B. cylindrica* roots (BCR), *B. gymnorrhiza* roots (BGR), *C. decandra* roots (CDR), *R. apiculata* roots (RAR), *R. mucronata* roots (RMR), *B. cylindrica* bark (BCB), *B. gymnorrhiza* bark (BGB), *C. decandra* bark (CDB), *R. apiculata* bark (RAB), *R. mucronata* bark (RMB), *B. cylindrica* leaves (BCL), *B. gymnorrhiza* leaves (BGL), *C. decandra* leaves (CDL), *R. apiculata leaves* (RAL), *R. mucronata leaves* (RML), *B. cylindrica hypocotyl* (BCH), *B. gymnorrhiza hypocotyl* (BGH), *C. decandra fruit* (CDF), *R. apiculata fruit* (RAF), *and R. mucronata fruit* (RMF).

2.3 Antimicrobial Activity

The methanol extract was tested for antimicrobial activity against *S. aureus* (gram-positive bacteria), *E. coli* (gram-negative bacteria), and *C. albicans* (a common opportunistic fungal species that causes skin infections). Hudzicki's disc diffusion method was used to evaluate antimicrobial activity [42]. Microbial suspensions were prepared following McFarland standards. Each petri dish received 1 mL of microbial suspension. Then, 25 mL of Mueller Hinton Agar (MHA) media was added to test antibacterial activity, and 25 mL of Sabouraud Dextrose Agar (SDA) media was added to test antifungal activity. Several disks containing the extract concentration (50% w/v), an antibiotic disk (positive control), and a negative control disk (containing methanol solvent) were placed on the surface of the media. Positive controls for the test include the antibiotics gentamicin (on *E. coli* and *S. aureus* bacterial colonies) and ketoconazole (on *C. albicans* fungal colonies). The samples were next incubated at 37 °C for 24 hours. Each antimicrobial test was repeated three times for each sample. The inhibition zone without any bacterial or fungal growth in the media is measured as an indication of antimicrobial activity.

2.4 Measurement of total tannin content

The total tannin content (TTC) was determined using the Folin Ciocalteu colorimetric method with the standard tannic acid compound, as described by Ahad et al. 2021 [43]. The sample solution was made by weighing 5 mg of the dry extract sample and dissolving it in up to 5 ml of methanol. Then, from this solution, take 0.2 mL, add Folin Ciocalteu (1 mL), 35% Na_2CO_3 solution (2 mL), and distilled water (16.8 mL). The solution was shaken until homogeneous and then incubated at room temperature (25 °C) for 30 minutes. The test solution has a concentration of 10 g/ml in this test, and the absorbance is calculated using a UV-vis spectrophotometer. Tannic acid standard solutions are made by weighing 10 mg of tannic acid and dissolving it in 20 ml of distilled water. After that, take 1, 1.5, 2, 2.5, 3, 3.5, and 4 ml of the solution and add distilled water to exactly 5 ml (solution concentrations of 100, 150, 200, 250, 300; 350; and 400 µg/ml). Following that, 0.2 mL of the concentration solution was mixed with 1 mL of Folin Ciocalteu, 2 mL of 35% Na_2CO_3 solution, and 16.8 mL of distilled water. After 30 minutes of incubation at room temperature (25 °C). A tannin content calibration curve was created using a tannic acid solution as a standard (1 - 4 µg/ml). The absorbance of samples and standard solutions was measured with a UV-Vis spectrophotometer at 725 nm, and the tannin content was calculated in milligrams of tannic acid equivalent per gram of extract (mg TAE/g) of dry extract.

2.5 Data Analysis

We rigorously applied statistical procedures to ensure the reliability and validity of our findings [44, 45]. Values are expressed as means (standard error). The Shapiro-Wilk test was used to ensure that data on tannin content and antimicrobial activity were normally distributed. Statistical significance was determined using one-way analysis of variance (ANOVA). Comparison of individual means resulted from Duncan's test using the computer program SPSS for Windows, version 21. The Pearson correlation coefficient was calculated using a 95%

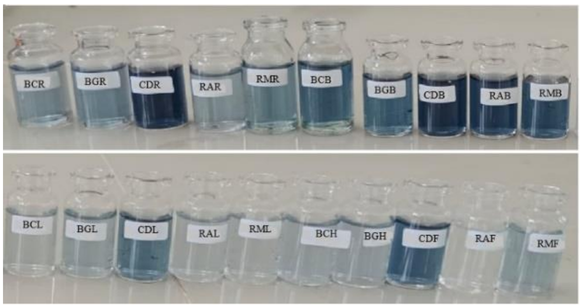

Figure 1. The visual appearance of the Rhizophoraceae methanol extract tannin test (sample code refer to section 2.2).

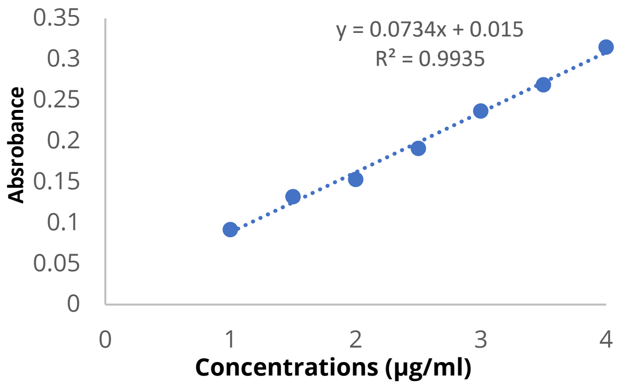

Figure 2. Calibration curve for tannic acid standards.

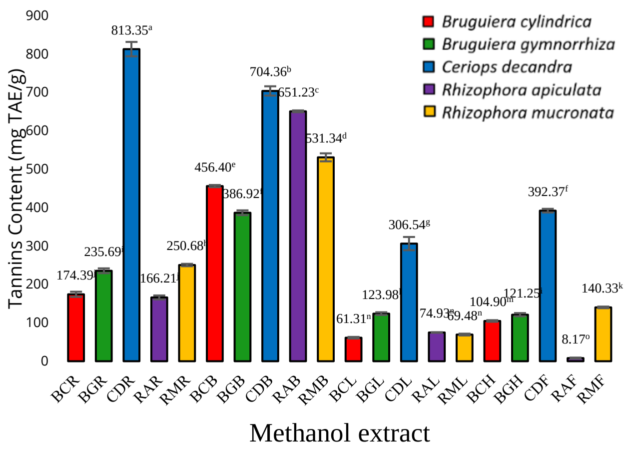

Figure 3. The total tannin content of Rhizophoracea methanol extract.

significance level on the tannin and antimicrobial content data. Each sample was tested in triplicate.

3. Results and Discussion

3.1 Total Tannins Content (TTC)

The total tannin content was determined using the Folin Ciocalteu colorimetric method proposed by Ahad et al. [43] with tannic acid as a standard. The tannin test solution with extract concentration is depicted in Figure 1. The color of the solution changed from yellow, which came from Folin Ciocalteu to blue after 30 minutes of incubation with 35% Na_2CO_3 solution and distilled water, indicating the presence of tannin. The total tannin content of Rhizophoraceae methanol extract was calculated using a tannic acid calibration curve as a standard. Tannic acid's standard curve is displayed in Figure 2.

Tannin compounds are polyphenolic compounds with a complex structure found in plants, the content of which varies between plant species [46]. The total tannin content of twenty Rhizophoraceae plant parts is shown in Figure 3. Total tannin content varied from 813.35 ± 18.72 mg TAE/g to 8.17 ± 1.36 mg TAE/g ($p < 0.05$). The roots of *C. decandra* (CDR) contained the most tannin compounds, while the fruit of *R. apiculata* (RAF) contained the least. In In the three highest orders, variation increases RAB > CDR > CDB.

The values of tannin content in several plant parts measured were different from those reported in the literature [43, 47]. The bark of *B. gymnorrhiza* (BGR) showed a TTC of 98.01 ± 1.94 mg CAE/g and the fruit of *B. gymnorrhiza* (BGF) 97.85 ± 4.41 mg CAE/g [47] in the water extract using standard caffeic acid, which was lower than the results of this study BGR 235.69 ± 6.29 mg TAE/g and BGF 121.25 ± 4.16 mg TAE/g. Using the same standards as in this study, TTC 18.37 mg TAE/g was discovered in the ethanol extract of *C. decandra* leaves (CDL) and also revealed a lower content than that found in this study CDL 306.54 ± 17.25 mg TAE/g [43]. Variations in total tannin content can be caused by a variety of factors, including the type of solvent used and the standards used [48]. Variation can also be attributed to differences in phytogeography, soil conditions, and seasons when plants are harvested, allowing plants to modify their phenolic compound and phytochemical content [49, 50]. Rhizophoraceae plants are mangrove halophytes that thrive in high-salinity environments. According to the findings, the soil in the Langsa Mangrove Forest area of Aceh at the research site has a clay and dust texture and a very low content of soil elements N, P, and K, which is an extreme habitat condition for plant growth. However, the results of this study revealed a high content of secondary metabolites of tannins, particularly in the bark of each Rhizophoraceae species. High tannin metabolites are a physiological adaptation response of plants in defense of reactive oxygen species (ROS) and oxidative stress, with salt stress stimulating their accumulation [51].

High levels of tannin (>350 mg TAE/g) are found in the bark of the five Rhizophoraceae species that contribute the most tannin from plant parts. The bark contains more tannins than the roots (except CDR), leaves, and fruit/hypocotyl. Muhayyidin reported that *R. mucronata*

Table 1. The antibacterial and antifungal activity of Rhizophoraceae methanol extract.

Extract	Inhibition zone diameter (50% extract concentration) (mm)		
	Staphylococcus aureus	***Escherichia coli***	***Candida albicans***
BCR	8.63 ± 0.69^{abc}	-	-
BGR	11.17 ± 1.17^{de}	-	-
CDR	13.74 ± 0.74^{f}	-	-
RAR	10.40 ± 0.92^{cd}	-	-
RMR	10.24 ± 0.42^{cd}	-	9 ± 0.03
BCB	13.08 ± 0.64^{ef}	-	-
BGB	13.87 ± 2.15^{f}	-	-
CDB	9.99 ± 1.20^{cd}	-	-
RAB	14.78 ± 0.90^{f}	-	-
RMB	11.68 ± 1.95^{de}	-	-
BCL	6.64 ± 0.80^{a}	-	-
BGL	11.24 ± 1.24^{de}	-	-
CDL	14.44 ± 1.18^{f}	-	-
RAL	10.34 ± 2.35^{cd}	-	-
RML	9.91 ± 1.17^{cd}	-	-
BCH	-	-	-
BGH	10.39 ± 0.93^{cd}	-	-
CDF	15.02 ± 0.32^{f}	-	-
RAF	7.76 ± 0.44^{ab}	-	-
RMF	8.92 ± 0.38^{bc}	-	-
Control	$22.55 \pm 0.04^{g*}$	19.867 ± 0.41	27.03 ± 0.51

Note. The bacterial test control was gentamycin, and the antifungal test control was ketoconazole (different letters on the same row indicate statistically different results at $p < 0.05$).

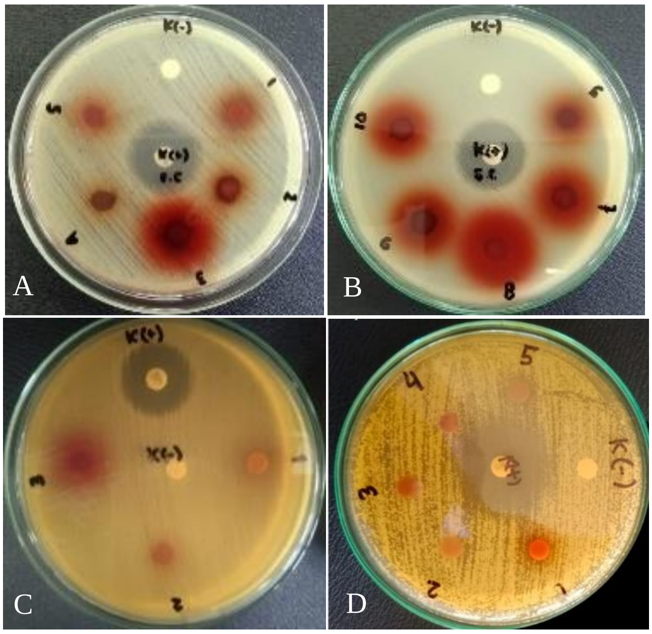

Figure 4. Rhizophoraceae methanol extract activity against A-B) *S. aureus*, C) *E. coli*, and D) *C. albicans*.

bark extract produced 76% condensed tannin [37]. Meanwhile, Hilmi et al. 2021 reported that the highest tannin range was found in mangrove bark, with the bark of *R. apiculata* and *R. mucronata* 386.60 – 460.38 kg/tree (tree diameter > 10 cm) having the highest total tannin range [52]. The main source of tannin is mangrove bark. Because the bark and stem contain cellulose, hemicellulose, lignin, and extractive materials, the tannin content in the bark is higher than in other parts [53]. Tannins are also found in areas of tree growth, specifically the phloem and xylem layers between the cortex and epidermis, indicating their influence on tissue growth in these areas [31].

3.2. Antibacterial Activities

The disk diffusion method was used in this study to assess the antimicrobial potential of Rhizophoraceae methanol extract. The antibacterial activity of Rhizophoraceae methanol extract was tested against the gram-positive bacteria *S. aureus,* the gram-negative bacteria *E. coli,* and the fungus *C. albicans,* and the results are shown in Table 1. *S. aureus* was significantly inhibited by a 95% methanol extract of Rhizophoraceae. However, no inhibitory activity was observed against *E. coli* bacteria or *C. albicans* fungi (95%). Figure 4 depicts the extract's activity against bacteria and fungi. The methanol extract demonstrated moderate to strong antimicrobial activity against *S. aureus* at a concentration of 50% extract in the inhibition zone range of 6.64 ± 0.80 mm - 15.02 ± 0.32 mm.

The strongest antibacterial activity was found in *C. decandra* fruit extract (CDF), *R. apiculata* bark (RAB), and *C. decandra* leaves (CDL). Methanol extract is ineffective against *E.coli* bacteria and *C. albicans* fungus, but it is extremely effective against *S. aureus*, as evidenced by several studies that follow the same pattern [54, 55].

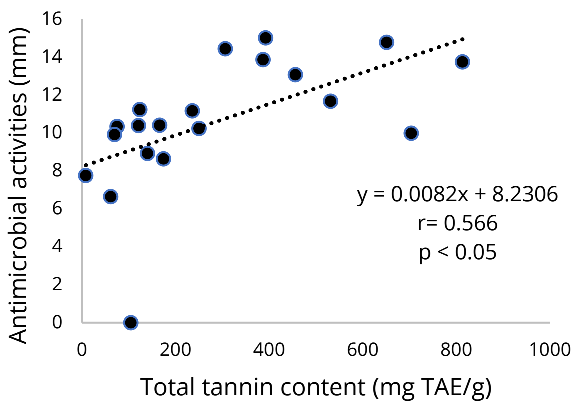

Figure 5. Linear correlation between the amount of total tannins (TAE) and antimicrobial activities (*S. aureus*). Pearson correlation, co-efficient correlation r = 0.566, significance at $p < 0.05$, linear equation y=0.0082x+ 8.2306.

Differences in the response of plant extracts to gram-positive and gram-negative bacteria have been previously reported, with gram-negative bacteria being more resistant than gram-positive bacteria. This is due to the fact that gram negatives have a double lipopolysaccharide membrane, which makes the cells impermeable to external compounds and can limit the diffusion of hydrophobic compounds through the lipopolysaccharide layer [40, 56, 57]. The outer layer of gram-positive bacterial membranes is made of peptidoglycan, which is permeable but inefficient against certain metabolite compounds, allowing it to be easily penetrated [55]. Furthermore, the high tannin content of the extract is linked to tannin's antibacterial properties, as tannin is considered a potent antimicrobial agent among many other phytocompounds.

Tannins inhibit bacterial growth, prevent biofilm formation, reduce toxin production and release, and kill bacterial cells directly [58]. Tannins' antibacterial activity is explained by their ability to pass through the bacterial cell wall to the internal membrane, disrupting cell metabolism and peptidoglycan synthesis, which causes cell wall formation to be imperfect. In Gram-positive bacteria, tannin activity is rapid [59]. The activity of the extract demonstrates resistance to the *C. albicans* species, a type of yeast cell. This resistance may be attributed to the capsule it contains [40].

3.3. Correlation between Tannin Content and Antibacterial Activity

Pearson correlation analysis was used to determine whether the tannin content of Rhizophoraceae methanol extract affects the antimicrobial activity of *S. aureus.* Data on total tannin content and *S. aureus* antimicrobial activity of the methanol extract was used for correlation analysis. A summary of the relationship between TTC and the inhibitory diameter of *S. aureus* bacteria is shown in Figure 5.

The Pearson correlation coefficient (r = 0.566) indicates a moderate correlation with a 95% confidence level of $p < 0.05$. A positive correlation means that as TTC levels increase, the average inhibitory activity increases. This correlation is consistent with previous research that discovered a moderate correlation between tannin polyphenols and antibacterial activity [60]. Based on this relationship, it explains that tannins are not the only compound with antimicrobial activity. There are other antimicrobial metabolite compounds including alkaloids, phenolics, flavonoids, terpenes, quinones, and resins [61–63]. This fact can provide additional insight into the relationship between compound content and antimicrobial response.

4. Conclusions

This paper briefly describes the total tannin content and antimicrobial activity and their correlation from the methanol extract of Rhizophoraceae from Langsa Aceh, Indonesia. Rhizophoraceae methanol extract has been shown to have high tannin levels as well as antimicrobial activity against *S. aureus* bacteria. The total tannin content ranged between 8.17 ± 1.36 mg TAE/g and 813.35 ± 18.72 mg TAE/g. The extract's antibacterial activity against the gram-positive bacteria *S. aureus* ranged from moderate to strong in the inhibition zone diameter range of 6.64 ± 0.80 mm - 15.02 ± 0.32 mm. Among the five Rhizophoraceae species, *C. decandra* is considered the best. The stem bark has the highest tannin content (813.35 ± 18.72 mg TAE/g), and the fruit has the best antimicrobial activity against *S. aureus* (15.02 ± 0.32 mm). The correlation between total tannin and antibacterial content is moderate, with a Pearson correlation coefficient of r = 0.566, $p< 0.05$. Selected samples for various purposes can be developed into a variety of pharmaceutical and health products.

Author Contributions: Conceptualization, methodology, validation, formal analysis, investigation, I.I., B.G., K.H., and D.D.; software, resources, I.I. and D.D.; data curation, I.I and B.G. writing—original draft preparation I.I, D.D; writing—review and editing I.I, B.G., K.H., and D.D.; visualization I.I; Supervision B.G., K.H., and D.D.; project administration, I.I.; funding acquisition, I.I. All authors have read and agreed to the published version of the manuscript.

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