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Antibacterial Activity of *Sinularia sp*. Against *Escherichia coli* and *Staphylococcus aureus*: Meta-Analysis and Systematic Review

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ABSTRACT

The Ocean covers more than 70% of Indonesian archipelago, providing various bioactive compounds with great potential, however, it is relatively underexplored *Sinularia sp*, a genus of soft corals, exists at the coral reefs of Indo-Pacificocean. Several recent studies have shown that *Sinularia sp*. contains various types of secondary metabolites with antiviral, antibacterial, anticancer, and antimalarial properties. In addition, further exploration and discovery of novel antibacterials that are effective against wide spectrum of bacteria is of great interest, especially to combat the emerging antibiotic resistance. The aim of this study is to evaluate the potential of antibacterial activity from *Sinularia sp* against *E. coli* and *S. aureus* which in turn, may provide an alternative antibacterial to overcome the emerging antibiotic resistance. This study was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement to measure the effect size of the antibacterial activity from *Sinularia sp*. against *E. coli* and *S. aureus*. Estimated value of the effect size obtained is 2.52 (CI 95% 0.98 to 4.06) and 5.97 (CI 95% 3.18 to 8.77) towards *E. coli* and *S. aureus*. When viewed as a whole, published data appears to show a positive antibacterial effect from *Sinularia sp*.

Keywords: *Sinularia sp*., antibacterial, *E. coli*, *S. aureus*, meta-analysis, antibiotic resistance.

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INTRODUCTION

One of the areas on earth that is relatively underexplored in terms of sources for natural products is the ocean. The ocean is the ecosystem and home to many organisms that contain unique chemical entities (Leal et al., 2019). The exploration of the ocean by humans to discover new organisms containing novel chemical substances is an ongoing process (Newman & Cragg, 2020; Ibrahim et al., 2023). One of the organisms of interest as a source for drug discovery efforts is soft corals (GEN, 2023; Liu et al., 2022). Soft corals are a type of invertebrate that live on coral reefs in the ocean or rocky areas in shallow water (Dobretsov et al., 2015). Soft corals have been shown to contain several

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substances, such as terpenoids, as a self-defense mechanism against external threats (Dobretsov et al., 2015; Katiandagho et al., 2019). *Sinularia sp*. is one of the soft corals from the Alcyoniidae family, which has more than a hundred species spread around East Africa to the West Pacific, though only a few of them have been studied (Katiandagho et al., 2019; Putra et al., 2021). Several studies have found that *Sinularia sp*. produces bioactive substances with various effects such as antibacterial, anti-inflammatory, antiviral, and anticancer properties (Dobretsov et al., 2015; Katiandagho et al., 2019; Putra et al., 2021; Tanod et al., 2018). Natural products have been of great interest as a source for drug candidate discovery, particularly in combating the growing problem of infectious diseases (Ziko et al., 2022). Antibiotic resistance is an emerging problem that requires innovation and new sources for novel antibiotics (Chopra et al., 2003). The investigation of *Sinularia sp*. can be one of the strategies in that effort.

Various classes of compounds have been identified in *Sinularia sp*., for example norcembranoids (C-4 Norcembranoids), sesquiterpenes, diterpenes, secosteroids, polyhydroxylated steroids, and polyamines (Katiandagho et al., 2019; Zubair et al., 2018). Several studies have previously identified, isolated, and elucidated terpenoid compounds with antibacterial activity. However, there is also a study that shows a limited antibacterial effect compared to pharmacological interventions. A study conducted by Putra et al. (2021) shows that a fraction of *Sinularia sp*. in ethyl acetate and methanol exhibits a low inhibition zone for *E. coli* (Putra et al., 2021).

This meta-analysis aims to address the potential of *Sinularia sp*. activity against *S. aureus* and *E. coli* to establish its effect size and whether the effect is significant or insignificant.

METHOD

Design. Systematic review and meta-analysis were conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.

Search Strategy. The search of studies was performed as comprehensively as possible, involving five databases, i.e., PubMed, ScienceDirect, ProQuest, Garuda, and Google Scholar, with defined inclusion criteria. The keywords used in the literature search were "*Sinularia sp*." OR "soft corals" AND "antibacterial" OR "antimicrobial." The unpublished studies were also searched through the repository or database of several universities, including AtmaLib; however, there were no relevant results. The search results were imported into Mendeley version 1.19.4 as a reference manager software, and the duplicates were removed. The PRISMA chart is presented in Figure 1.

Inclusion and Exclusion Criteria. The inclusion criteria for this study included 1) in vitro studies of *Sinularia sp*. with antibacterial activities published in the last 10 years (2012-2022), and 2) studies showing mean and standard deviation values. The exclusion criteria included 1) review articles, 2) in silico-based studies, and 3) studies without full text (unretrievable).

Extraction and Data Analysis. All authors contributed in every stage of the literature review by screening through the title and abstract, and screening through the full-text article for eligibility, including and excluding the articles. We used all the relevant studies which we were able to retrieve and tabulate the quantitative data in the form of mean and standard deviation values or r-values between *Sinularia sp*. and its antibacterial activities.

The software used for processing the articles was JASP version 16.3. Based on the calculation, several parameters were established, such as I2, which indicates the heterogeneity of the studies and which we used as the basis for fixed-effect model implementation. The result is statistically significant if the p-value is more than 0.05 in a confidence interval of 95%. The effect size for each study, overall effect, and standard error were visualized in the forest plot.

To avoid publication bias, we performed a bias analysis using a funnel plot. Furthermore, trim and fill analyses were conducted to identify the missing studies.

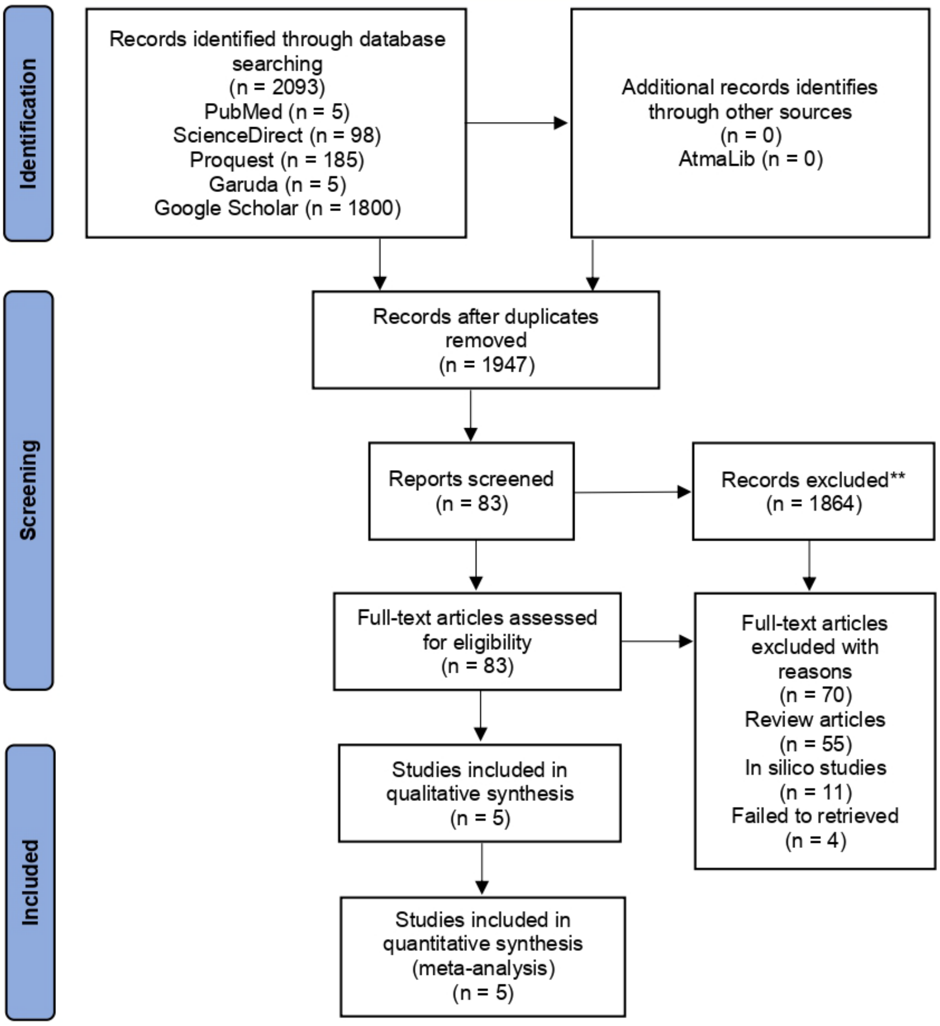


Figure 1. The PRISMA Flowchart of the meta-analysis and systematic review

RESULTS AND DISCUSSIONS

Study Selection. Literature search from electronic media resulted in 2,093 studies, from which 146 studies were eliminated due to duplication. After reviewing the title and abstract, 1,864 studies were removed, leaving 83 studies for full-text analysis. Based on inclusion and exclusion criteria, 70 articles were excluded. Exclusions were implemented on 55 review articles, 11 in silico-based studies, and 4 unretrievable articles. After exclusion, 5 articles met the eligibility of inclusion criteria for qualitative and quantitative analysis.

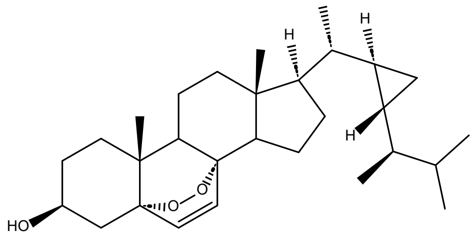
Study Characteristics. Data abstraction from 5 articles is presented in Table 1. Each article generated 24 coded studies for *E. coli* and 25 coded studies for *S. aureus*. Those coded studies might enhance the effect size of antibacterial activity from *Sinularia sp*.

Table 1. Data Abstraction

Ref	Author, year	Treatment	Outcome	Coded Study (N)
[1]	Katiandagho, et al. (2019)	Antibacterial activity test of ethanolic extract using maceration method, methanol-water fraction, n-hexane fraction, and chloroform fraction of *Sinularia sp*. were performed against *E. coli* and *S. aureus* using modified agar diffusion assay.	The study did not specify the bioactive compounds found in the extracts. The extract and fractions inhibits the growth of *E. coli* and *S. aureus* with moderate to strong inhibition.	1a-1d (4)
[2]	Tanod, et al. (2018)	Three extracts of *Sinularia sp*. (dichloromethane, ethyl acetate, and ethanol) initially prepared using maceration method, then were made at concentrations of 1, 10, 100, and 1000 mg/mL to test against *E. coli* and *S. aureus* using agar diffusion method.	The extracts from various solvents contain saponins, steroids, alkaloids and polyphenols. Dichloromethane extract, which contains saponin, steroid, and alkaloid, shows the highest activity against *E. coli* dan *S. aureus*.	2a-2h (8)
[3]	Zubair, et al. (2018)	Antibacterial activity test of methanolic extract of *Sinularia sp*. 1 and *Sinularia sp*. 2 acquired from maceration method were prepared at concentration of 1000 mg/mL, then the extracts were tested using agar diffusion method against *E. coli* and *S. aureus*.	The preliminary study shows that the extract contains terpenoids compound. The extract of *Sinularia sp*. 2 shows the highest diameter of inhibition zone, indicating a wide spectrum of antibacterial effect.	3a-3b (2)
[4]	Putra, et al. (2021)	Sample was extracted by maceration method using MeOH:CH_2Cl_2 (1:1) solvent. After removal of solvents, nine fractions of *Sinularia sp*. were prepared (n-hexane, n-hexane:ethyl acetate 8:2, n-hexane:ethyl acetate 6:4, n-hexane:ethyl acetate 4:6, n-hexane:ethyl acetate 2:8, ethyl acetate, ethyl acetate:methanol 8:2, ethyl acetate:methanol 6:4, ethyl acetate:methanol 2:8) to test for their antibacterial activities at concentration of 250 ppm.	Two steroid compounds were isolated and characterized. Those nine fractions showed antibacterial activity which was demonstrated by the moderate to strong inhibition zone. The most potent activity was shown from n-hexane:ethyl acetate 4:6 fraction.	4a-4i (9)
[5]	Dobretsov, et al. (2015)	Extracts of *Sinularia sp*. by maceration using methanol and chloroform solvents were tested against *E. coli* and *S. aureus* using disc diffusion method.	Two compounds of long chain carboxylic acid esters were acquired. Test extracts show inhibition activity against *E. coli* and *S. aureus* with moderate to strong inhibition zone.	5a-5b (2)

Antibacterial Activity of *Sinularia sp.* The differences between studies are 1) the species of *Sinularia sp*., 2) extraction/fractionation solvent, 3) concentration of extract/fraction, 4) *E. coli* and *S. aureus* strains, and 5) test conditions.

The study conducted by Putra et al. (2021) showed that *Sinularia sp.* fractioned with n-hexane: acetate 40:60 possesses the highest potency of antibacterial activity compared to other fractions. At a concentration of 1000 mg/mL, that fraction shows inhibition zone diameters of 11.98 ± 0.74 and 14.53 ± 0.51 mm for *E. coli* and *S. aureus*, respectively. This antibacterial property can be attributed to the presence of two isolated steroid compounds, which are (22R,23R,24R)-22,23-methylene-24-methylcholest-6-en-5α,8α-epidioxy-3β-ol and 5α,8α-Epidioxy-24(R)-methylcholesta-6,22-dien-3α-ol (Putra et al., 2021).



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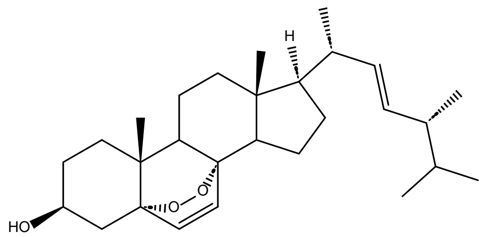

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Figure 2. Two steroids compounds (22R,23R,24R)-22,23-methylene-24-methylcholest-6-en-5α,8α-epidioxy-3β-ol (1) and 5α,8α-Epidioxy-24(R)-methylcholesta-6,22-dien-3α-ol (2) which are considered responsible for the antibacterial property of *Sinularia sp*.

On the other hand, a study by Tanod et al. (2018) reported that dichloromethane shows higher antibacterial activity than ethyl acetate and ethanol fractions. At a similar concentration, the dichloromethane fraction of *Sinularia sp*. demonstrated inhibition zone diameters of 13.10 ± 1.45 and 14.73 ± 0.75 mm for *E. coli* and *S. aureus*, respectively (Tanod et al., 2018). Both studies demonstrated that *Sinularia sp*. fractions are more effective in inhibiting the growth of *S. aureus* compared to *E. coli* (Putra et al., 2021; Tanod et al., 2018). The fractions were tested for their zoochemical compositions and it was discovered that each fraction contains classes of major components, although the compounds were not specified further. Dichloromethane fraction was reported to contain saponin, steroid, and alkaloid. Ethyl acetate fraction contains polyphenol, steroid, and alkaloid. While ethanol fraction was reported to possess polyphenol compounds. This finding is consistent with the study conducted by Jia et al. 2005 and Handayani et al. 1997 which investigated the role of these compounds for *Sinularia sp*., particularly for the protection against predators (Jia et al. 2005 and Handayani et al. 1997).

Other findings by Katiandagho et al. (2019) and Zubair et al. (2018) show a similar inhibition activity from methanol fraction of *Sinularia sp*. against *E. coli* and *S. aureus* with the diameter of inhibition zone around 12.33-15.80 mm.

Dobretsov et al. (2015) reported somewhat different results. *Sinularia sp*. extracts displayed greater inhibition activity on the growth of *E. coli* rather than on *S. aureus*. However, the results are still within the range of moderate to strong inhibition with diameters of 9-12 mm ($p < 0.001$) (Dobretsov et al., 2015). Two compounds which are thought to provide the antibacterial activity include the two compounds of long chain carboxylic acid esters, which are hexadecyl palmitate and hexadecyl stearate, although the study disclaimed that the mechanism of action of those long chain carboxylic acid esters were not elucidated.

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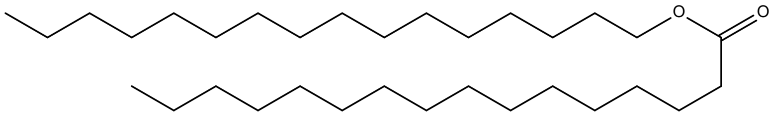

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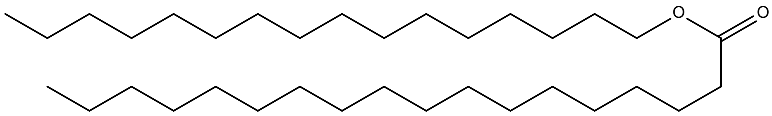

B

Figure 3. Two compounds of long chain carboxylic acid esters hexadecyl palmitate (A) and hexadecyl stearate (B) which are active against bacterial fouling.

Differences in sensitivity towards antibacterial agents between Gram-positive and Gram-negative bacteria are affected by the structure of the cell wall, such as the thickness of peptidoglycan, the activity of autolytic enzymes, and the cross-linking behavior. Gram-negative bacteria such as *E. coli* possess an extra outer membrane integrated with porin that facilitates the diffusion of low molecular weight hydrophilic molecules (Jawetz et al., 2008). Gram-negative bacteria are generally more sensitive towards polar antibacterials, while Gram-positive bacteria are more susceptible to nonpolar antibacterials (Brannen & Davidson, 1993).

The significant activity towards both types of bacteria proved that *Sinularia sp*. possesses a wide spectrum of antibacterial activity.

Meta-analysis. We utilized JASP software v.0.16.3 to perform the meta-analysis with a random effect method. This method is more suitable for this study because of the high variance and variables that distinguish between the pooled studies.

This meta-analysis focuses on the general study design by including studies with an experimental design against the most common Gram-negative bacteria, *E. coli*, and Gram-positive bacteria, *S. aureus*.

Sinularia sp. demonstrates a statistically significant antibacterial activity compared to the control ($p < 0.001$) with Cohen's d value of 2.52 (CI 95% 0.98 to 4.06) and 5.97 (CI 95% 3.18 to 8.77) towards *E. coli* and *S. aureus*, respectively (Figure 4). This result indicates a strong antibacterial effect of *Sinularia sp*. There is no negative effect nor effect favoring control found from the antibacterial test of *Sinularia sp*.

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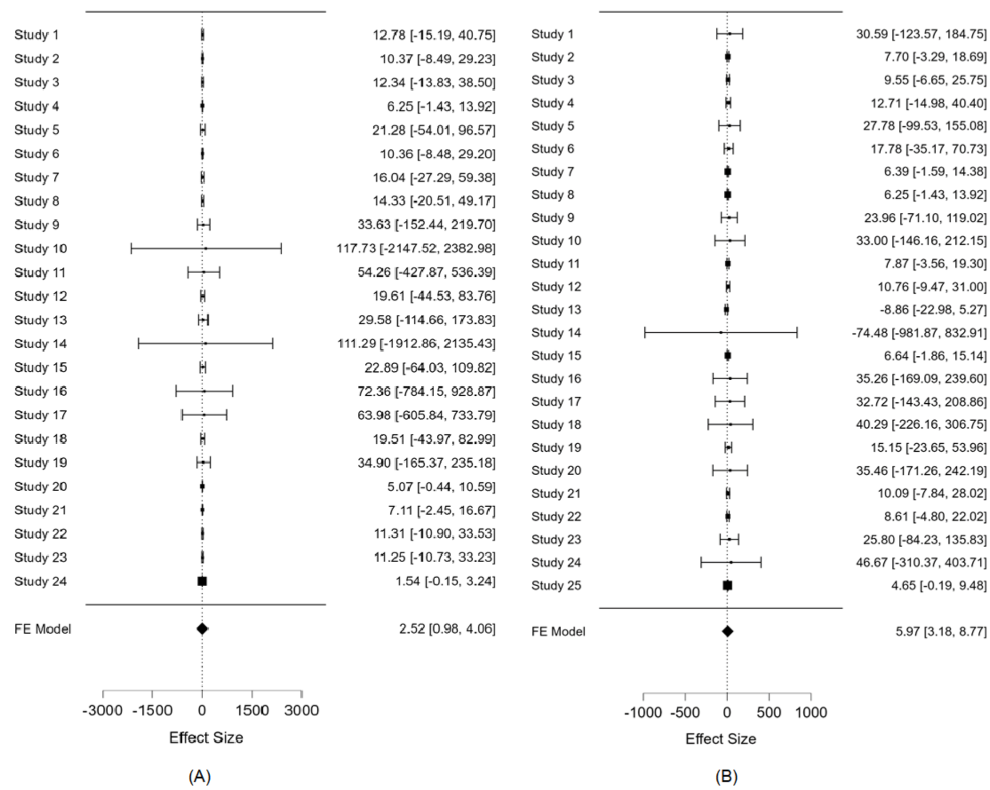

Figure 4. The forest plot depicting the summary of estimated effect size from the pooled studies included in this meta-analysis (A) towards *E. coli*, and (B) towards *S. aureus*.

Bias analysis. The potential of bias in the articles was assessed using a funnel plot (Figure 5). The funnel plot, however, indicates a bias shown by the asymmetric plot which tends to assemble on the positive side. It occurs commonly due to several limitations in meta-analysis, for example, the unfound unpublished studies and language limitations. Other limitations include the unavailable data of negative control in the whole study (Page et al., 2020).

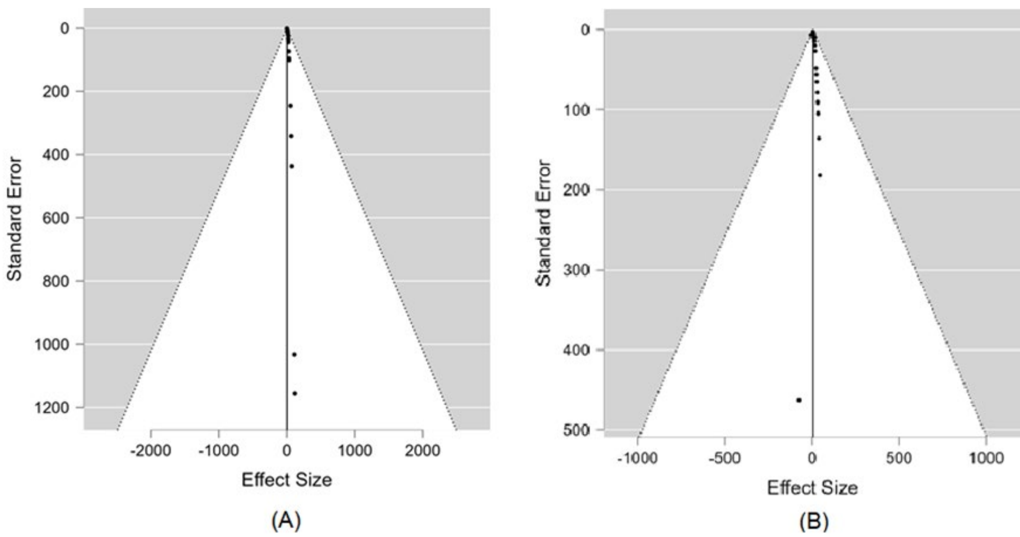

Figure 5. The funnel plot showing the bias potential in this meta-analysis: (A) data for *E. coli*, (B) data for *S. aureus*

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The trim-and-fill analysis was conducted using the JASP program to identify the missing studies and to estimate the effect size when the bias does not occur. In the produced plot, the missing studies are represented by the eight white dots (Figure 6), which are needed to eliminate the bias.

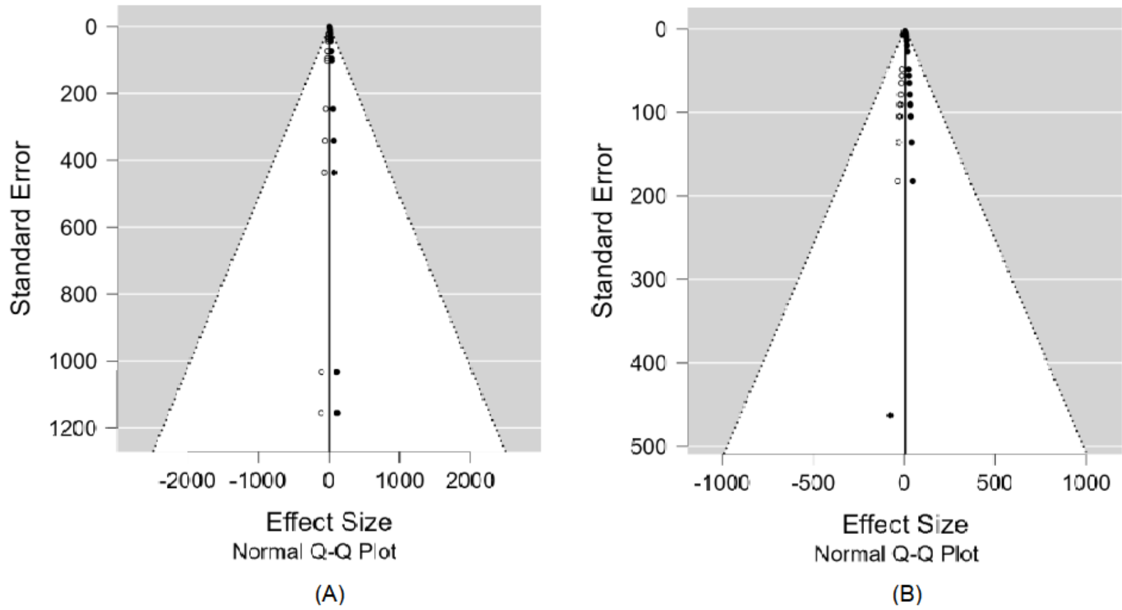

Figure 6. The trim-and-fill analysis: (A) data for *E. coli*, (B) data for *S. aureus*

We have attempted to search all the relevant published and unpublished studies that meet the inclusion criteria from the defined search engines. The search for unpublished studies is quite challenging in this study, and the fact that there are only five published studies used in this meta-analysis indicates the scarcity of experimental studies investigating *Sinularia sp*. However, the trim-and-fill analysis shows that there is no significant drop in the effect size when the missing studies are included. The effect size shifts slightly from 2.52 to 2.46 for *E. coli* and from 5.97 to 5.89 for *S. aureus*.

The small shift proves that the conclusion generated from this study is valid and reliable. However, the missing studies are still necessary to figure out whether there is another variation in the effect of *Sinularia sp*.

We are convinced that the number of studies about *Sinularia sp*. will grow in the future to fill up the limitations in the present study.

CONCLUSION

Most studies show the antibacterial properties of *Sinularia sp*. towards both Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus*, which is represented in the forest plot and the overall effect size. The effect sizes generated in this study are 2.52 (CI 95% 0.98 to 4.06) and 5.97 (CI 95% 3.18 to 8.77) towards *E. coli* and *S. aureus*, respectively. This meta-analysis highlights the potential of *Sinularia sp*. as one of the sources for drug discovery of antibacterials, which, in turn, may provide an alternative solution to the emerging antibiotic resistance.

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