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Anti-Inflammatory Effect of *Phyllanthus niruri*: A Meta-Analysis

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Abstract. Many studies report on the role of *Phyllanthus niruri* as an anti-inflammatory and there are variations in the results in every research, so a meta-analysis study is needed to obtain accurate results. This study aims to conduct a meta-analysis of the effect of *Phyllanthus niruri* as an anti-inflammatory. This study uses a meta-analysis research design with data collection from 12 databases. The screening process was carried out according to PRISMA, then analyzed using Comprehensive Meta-Analysis Version 3 (CMA3). Funnel plot and Egger's test were used as methods to measure publication bias. The articles used were 438 articles from various research journals. After going through screening, as many as 5 articles were eligible to be incorporated in the meta-analysis. Data were analyzed using a random effect model to calculate standardized means difference (SMD). The cytokines analyzed were TNF-α and IL-6 because both were discussed in at least 3 studies. *Phyllanthus niruri* affected the reduction of inflammatory cytokines with an effect size of SMD on TNF-α is -17,611 (95% CI -29,347, -5,875; p=0.003) and IL-6 is -3,263 (95% CI -5,403, -1,222; p = 0.003). The results of this meta-analysis strengthen the concept/theory that *Phyllanthus niruri* has the potential as an anti-inflammatory by reducing the inflammatory cytokines TNF-α and IL-6. These results strengthen the scientific basis for the use of *Phyllanthus niruri* as a supplement for the community to prevent inflammation.

Keywords: effect size, IL-6, inflammatory cytokines, standardized means difference, TNF-α

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INTRODUCTION

Chronic inflammation causes serious diseases such as cardiovascular disease, chronic kidney diseases, neurodegenerative disorders, diabetes mellitus, liver disorders, and autoimmune disorders (Furman et al., 2019). Inflammation is usually characterized by calor (hotness), rubor (redness), tumor (enlargement), dolor (soreness), and function laesa (disappearance of tissue function). These symptoms are caused by increased blood flow (vasodilation) and increased vascular permeability (movement of proteins, plasma fluids, and inflammatory cells from the blood vessels into the tissues), resulting in edema (Efron, 2017).

Anti-inflammatory drugs are divided into two groups, namely steroids and non-steroids. Anti-inflammatory drugs prevent prostaglandin release in damaged tissue (Bokti & Saputri, 2018). NSAIDs (non-steroidal anti-inflammatory diseases) have side effects of complications in the gastrointestinal, cardiovascular, liver, kidneys, cerebral, and lungs (Bindu et al., 2020). There is a need for alternatives to NSAIDs that are safe and have fewer side effects. Many studies confirm the role of herbal plants as anti-inflammatories (Ghasemian et al., 2016), one of which is *Phyllanthus niruri*.

The *Phyllanthus niruri* plant is widely used by the people of Central Sulawesi as a medicine for diarrhea, fever and cough (Ifandi et al., 2016). The results of the phytochemical tests showed that the methanol extract of *Phyllanthus niruri* contained alkaloids, flavonoids, tannins, saponins, and triterpenes (Sutrisna et al., 2019). Meniran leaves (*Phyllanthus niruri* L.) are effective in accelerating wound healing due to contamination because they contain triterpenoids. Triterpenoids can increase the formation of skin collagen and contain essential oils as an antibacterial effect, providing a pharmacological effect on wound healing as an anti-inflammatory agent (Couto et al., 2013). *Phyllanthus niruri* extract has been shown to decrease the expression of inflammatory mediators such as TNF-α, NF-KB, IL-6, IL-8, IL-10, and COX-2 (Al Zarzour et al., 2018). However, a research conducted by Ezzat et al. (2020) showed an increase in IL-6 cytokines after being given *Phyllanthus niruri* intervention.

Many studies report on the role of *Phyllanthus niruri* as an anti-inflammatory, and some others provide pro-inflammatory effects, so the results of a combined study are needed to draw the right conclusions. The method used for this study is known as a meta-analysis (Gurevitch et al., 2018). The meta-analysis is a statistical method that combine the results of quantitative research that has been published to produce conclusions from certain empirical data. This meta-analysis can specifically be used to analyze the central trend and variations of the data, and to correct errors and biases in research (DerSimonian & Laird, 2015). This study aims to conduct a meta-analysis of the effect of *Phyllanthus niruri* as an anti-inflammatory. These results strengthen the scientific basis for the use of *Phyllanthus niruri* as a supplement for the community to prevent inflammation

METHODS

Data mining

The data source for this research is primary data derived from literature from international journals obtained through online searches on the PubMed database, Scopus, Springerlink, Pro quest, Brill, Taylor & Francis, EBSCO, J Gate, Science Direct, Sage Journal, GARUDA, and Emerald insight. The article exploriation was limited to English-language research and was carried out within the last 10 years, that is 2014-2023.

The search keywords used are ((inflammation[MeSH Terms]) OR (inflammation) OR (inflammatory)OR (proinflammatory) OR (C-reactive protein[MeSH Terms]) OR (C-RP)) OR (CRP) OR (interferons[MeSH Terms]) OR (Interferons) OR (Interferon) OR (Interleukin-6[MeSH Terms]) OR (Interleukin-6) OR (Interleukin 6)) OR (IL-6) OR (IL6) OR (Tumor Necrosis Factor-alpha[MeSH Terms]) OR (Tumor Necrosis Factor-alpha) OR (TNF-alpha) OR (Tumor Necrosis factor α) OR (TNF-α) OR (Tumor Necrosis factor) OR (TNF) OR (TNF alpha) OR (TNF α) OR (Tumor necrosis factor α) OR (Interleukin-8[MeSH Terms]) OR (Interleukin-8) OR (Interleukin 8) OR (IL-8) OR (IL 8) OR (IL8) OR (Interleukin-10[MeSH Terms]) OR (Interleukin-10) OR (Interleukin 10) OR (IL 10) OR (IL10) OR (IL-10) OR (Interleukin-1[MeSH Terms]) OR (Interleukin-1) OR (Interleukin 1) OR (IL 1) OR (IL1) OR (IL-1) OR (NF-kappa B[MeSH Terms]) OR (NF-kappa B) OR (NF-kB) OR (endotoxins[MeSH Terms]) OR (endotoxins) OR (endotoxin) OR (lipopolysaccharides[MeSH Terms]) OR (lipopolysaccharides) OR (lipopolysaccharide) OR (LPS) OR (NF kappa B) OR (NF kB) OR (NFkB)) AND ((*Phyllanthus niruri*i[MeSH Terms]) OR (*Phyllanthus niruri*us[MeSH Terms]) OR (*Phyllanthus niruri*i) OR (*Phyllanthus niruri*) OR (P niruri) OR (P nirurii)).

Article selection and data extraction

The first step in the article selection process manually removes duplicate articles. Then, the article selected starts from the title, abstract, and read the contents of the article thoroughly. Articles incorporated in the meta-analysis study were selected based on inclusion and exclusion criteria which were arranged using the PICOS format (population, intervention, comparison, outcome) (Table 1).

Table 1. PICOS format with inclusion and exclusion criteria for article selection

Criteria	Inclusion	Exclusion
Population	Rat	Not a rat
Intervention	*Phyllanthus niruri*	Not a *Phyllanthus niruri*
Comparison	control group	Has no control group
Outcome	There is a relationship between *Phyllanthus niruri* and inflammatory cytokines	There is no relationship between *Phyllanthus niruri* and inflammatory cytokines
Study design	Experimental	Other than Experimental
Publication Year	2014-2023	Before 2014
Language	English	Not English
Data form	quantitative	Other than quantitative

All articles excluded must be given clear reasons for exclusion. After screening the abstracts, the complete manuscript of the articles was downloaded for further analysis. The screening and selection procedures for meta-analytical studies followed the PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*) guidelines (Page et al., 2021).

The raw data for each article is converted into a table of article characteristics which contains information about the title, researcher's name, year of publication, journal name, research design, research location, and database. Each article was also extracted into a critical appraisal table containing the name of the researcher, sample characteristics, number of samples, dosage, and research outcomes. The data presented in graphical form was extracted with the help of web plot digitizer software (Mani, 2018). The equations extracted in this type of research are the average, sample size, and standard deviation.

Publication Bias Test

This study uses the funnel plot technique and Egger's test to test for publication bias using Comprehensive Meta-analysis (CMA) software version 3. Funnel plots are graphical displays that show the distribution of estimated study sizes based on the strength of each study to identify publication bias (Kiran et al., 2017). In nothingness of bias and heterogeneity, the funnel plot will be funnel-shaped and centered symmetrically around the meta-analysis effect estimates. If the distribution of the effect sizes for each article is not symmetrical, it indicates that there is a publication bias in the variable studied (Zwetsloot et al., 2017). In addition to the funnel plot, egger's test was used to further ensure publication bias. If the intercept value is equal to zero (0), it indicates that publication bias does not affect the relationship of the variables tested.

Analysis Statistics

Every inflammatory cytokine must be discussed in at least 3 studies, to ascertain whether the effect is pro or counter (Meader *et al*., 2013). The software used in data analysis is Comprehensive Meta-Analysis V.3. According to Stanley dan Doucouliagos (2015), there are two statistical models for meta-analysis, that is the random effect model and the fixed effect model. The random effect style is used when the data is assumed to be quite heterogeneous ($I^2 > 50\%$). The fixed effect style is used when the data is considered homogeneous ($I^2 <50$). The data is said to be heterogeneous if the Chi-square value is $p <0.05$ and the I^2 value is > 50% (Borenstein et al., 2010). Graphs of data analysis results are presented in the form of forest plots. Forest plots are used for scanning and interpretation of evidence in meta-analyses, forest plot are a valid, fast and efficient tool (Verhagen & Ferreira, 2014).

RESULTS AND DISCUSSION

The total number of articles searched from 12 databases is 438 based on the criteria for publication in 2014-2023, in English and the research design is experimental. The results of the duplication analysis showed that 95 articles were excluded due to double, leaving 343 articles. As a result of the selection of titles, 259 articles were excluded because they did not use the intended keywords, namely *Phyllanthus niruri* and inflammatory cytokines.

Based on the selection of abstracts, 67 articles were excluded because they did not discuss the effect of *Phyllanthus niruri* on inflammatory cytokines. A total of three articles were excluded because they were published before 2014 (unreadable when searching for the initial article), two articles were excluded because the research design was non-experimental (systematic review), one article was excluded because the research subject was not a rat, 1 article was excluded because the intervention provided was not just *Phyllanthus niruri* alone but in combination with other plants, 4 articles were excluded because the abstract could not be accessed. Overall, 78 articles were excluded based on the selection of abstracts so only 6 articles passed the content selection stage.

As many as 1 article was excluded because the data presented was not in the form of a mean and standard deviation. The results of selection of content resulted in 5 articles that were acceptable to be incorporated in the meta-analysis. Details of the selection of articles compiled using the PRISMA diagram can be seen in Figure 1. Five research articles that eligible the criteria in this meta-analysis were then extracted into the article characteristics table (Table 2) and the critical appraisal table (Table 3). The research subjects of all articles are experimental rats that have been induced by inflammation such as diabetes mellitus, breast cancer, and diseases of the liver. Experimental animals were given *Phyllanthus niruri* intervention with certain doses to determine its effect on inflammatory cytokine levels, which in this study focused on TNF-α and IL-6 cytokines.

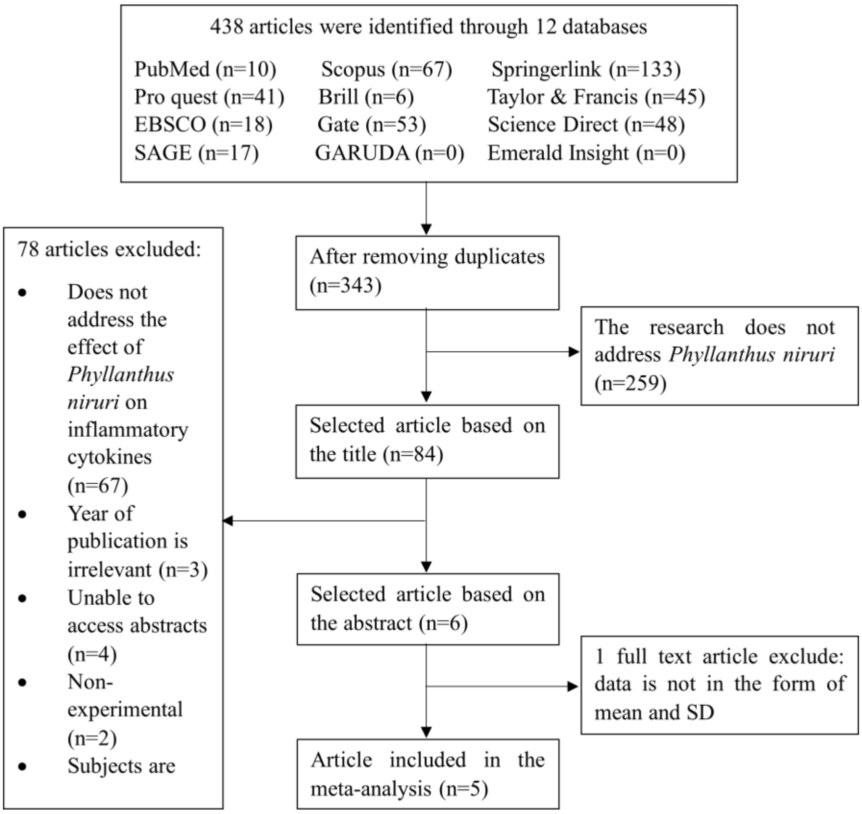

Figure 1. PRISMA diagram of article search results

Table 2. Characteristics of the article

No	Title	Researcher name (year)	Journal Name	Research design	Research sites	Database
1	*Phyllanthus niruri* leaves aqueous extract improves kidney functions, ameliorates kidney oxidative stress, inflammation, fibrosis and apoptosis and enhances kidney cells proliferation in adult male rats with diabetes mellitus	Giribabu et al. (2017)	Journal of Ethnopharmacology	experimental	India	J GATE
2	Suppression of hypoxia and inflammatory pathways by *Phyllanthus niruri* extract inhibits angiogenesis in DMBA-induced breast cancer mice.	Ramadhani et al. (2021)	Research in Pharmaceutical Sciences	experimental	Indonesia	SCOPUS
3	Adipocytokine regulation and antiangiogenic activity underlie the molecular mechanisms of therapeutic effects of *Phyllanthus niruri* against non-alcoholic fatty liver disease	Zarzour et al. (2018)	Nutrients	experimental	Malaysia	SCOPUS

4	In-depth hepatoprotective mechanistic study of *Phyllanthus niruri*: In vitro and in vivo studies and its chemical characterization	Ezzat et al. (2020)	PLoS ONE	experimental	Egypt	SCOPUS
5	Comparative Effects of *Phyllanthus niruri* and *Plantago major* in Carbon Tetrachloride Intoxicated Rats	Khamis et al. (2022)	Advances in Animal and Veterinary Sciences	experimental	Egypt	SCOPUS

Table 3. Critical appraisal articles

No	Researcher name (year)	Sample characteristics	Sample size	dose	Outcome			
					TNF-α		IL-6	
					Mean	SD	Mean	SD
1	Giribabu et al. (2017)	Adult male Wistar rats weighing 180-220g with diabetes mellitus	6/ group	0 mg/kg (control)	25.714	0.857	22.212	0.899
				200 mg/kg	14.449	0.857	15.737	0.629
				400 mg/kg	8.449	0.735	11.691	0.719
2	Ramadhani et al. (2021)	Female mice with DMBA-induced breast cancer	15/ group	0 mg/kg (control)	-	-	9.193	0.524
				500 mg/kg	-	-	7.928	0.586
				1000 mg/kg	-	-	7.866	0.679
				2000 mg/kg	-	-	7.280	0.524
3	Zarzour et al. (2018)	Male rats with non-alcoholic fatty liver disease	6/ group	control	31.277	3.191	2.042	0.377
				1000 mg/kg	21.702	1.596	0.691	0.071
4	Ezzat et al. (2020)	Male Wistar rats (200-250 g) with CCL4-induced liver toxicity	6/ group	control	-	-	11.800	4.21
				1000 mg/kg	-	-	49.000	4.65
5	Khamis et al. (2022)	Albino male rats (150-180 g) with CCl4-induced liver toxicity	10/ group	control	335.897	3.846	-	-
				500 mg/kg	174.359	5.128	-	-

Effect of *Phyllanthus niruri* on TNF-α

There are 3 articles analyzing the effect of *Phyllanthus niruri* on TNF-α, that is research by Giribabu et al (2017), Zarzour et al (2018), and Khamis et al (2022). The value of variation between studies (I^2) obtained was 94.2%, reinforced by a value of p = 0.000. This shows that the variation between studies is heterogeneous, so the analysis model used is the random effect model. The heterogeneity test is one of the tests for the basis for selecting a statistical meta-analysis model. The most frequently used heterogeneity test is the value of p (Chi-square), T^2 (Tau Square), and I^2 (I Square). These three methods are used to determine the variation that occurs between studies, including homogeneous or heterogeneous (Mohan & Adler 2019). If the results of the heterogeneity test show homogenous result, then the fixed effect model is used. Conversely, if the results of the heterogeneity test show heterogenous results, then a random effect model is used. The random effect model assumes that different study results may come from different populations with different responses to treatment. In general, the random effect model is more conservative and tends to show statistically significant results than the fixed effect model (Abuabara et al. 2012).

The results of data analysis shown in the forest plot (Figure 2) show that there is a significant relationship between *Phyllanthus niruri* and TNF-α with a value of p <0.05, p = 0.003, and a standardized mean difference value of -17,611 (95% CI -29,347, - 5,875). This shows that the *Phyllanthus niruri* intervention reduced the inflammatory cytokine TNF-α by 17,611 times compared to the control. Forest plots report the conclusions of several similar studies in an easy-to-understand graphical form. The forest plot consists of the effect size values for each study and the results of drawing conclusions or summary effects. The effect size of every study is reported in ‘square form’ with a certain confidence interval. The size of the square shows the value of the research weight (Verhagen & Ferreira 2014). The summary effect of the meta-analysis is reported in the form of a ‘diamond’ located at the lower end of the forest plot. The width of the diamond size shows the level of accuracy of the conclusions produced by the meta-analysis. The conclusions drawn can be said to be more accurate with the smaller of the diamond size. Conversely, the conclusions drawn can be said to be have a wider deviation value, with the wider of the diamond size (Verhagen & Ferreira 2014). In forest plots, the effects of every study and the combined effect estimates are illustrated by plots (points) with particular confidence interspaces. Thus, it can be seen that the effects of each study tend to be the same or differ from one study to the next (Monroe 2007).

The technique used to identify the presence of publication bias in this study is a funnel plot, and Egger's test was performed to further confirm the attendance of publication bias. The funnel plot shows an asymmetrical study distribution, where the research distribution is unequal between the right and left of the center line boundary (Figure 3). This indicates that there is a publication bias in the studies used. The results of the Egger's regression test founded P value <0.05, namely 0.005 and 0.01. This shows that there is an effect of publication on TNF-α due to *Phyllanthus niruri* administration.

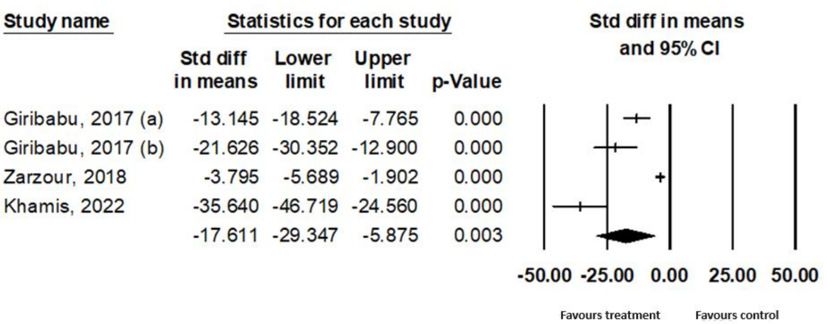

Figure 2. Forest plot of TNF-α cytokine meta-analysis results

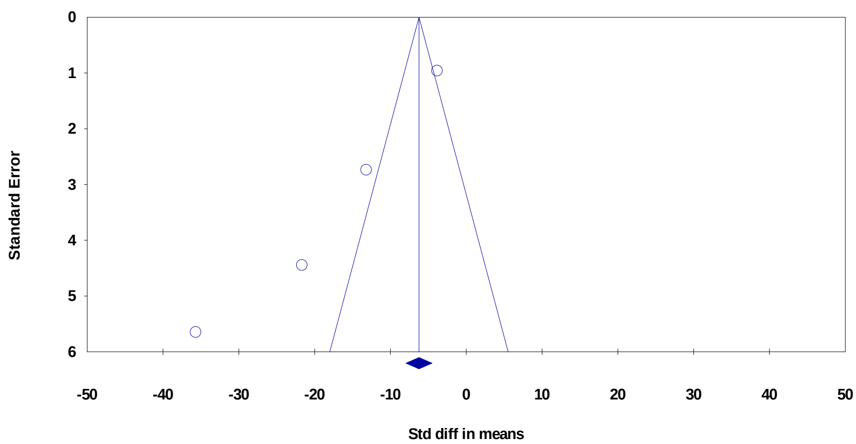

Figure 3. Funnel plot of the effect of *Phyllanthus niruri* on TNF-α. Diamond form illustrates the SMD effect size

Study name	Std diff in means	Lower limit	Upper limit	p-Value
	Statistics for each study			
Giribabu 2017 (a)	-8.346	-11.871	-4.820	0.000
Giribabu, 2017 (b)	-12.925	-18.219	-7.632	0.000
Ramadhani, 2021 (a)	-2.276	-3.194	-1.357	0.000
Rhamadani, 2021 (b)	-2.188	-3.093	-1.283	0.000
Rhamadani, 2021 (c)	-3.651	-4.819	-2.482	0.000
Zarzour, 2018	-4.980	-7.272	-2.689	0.000
Ezzat, 2020	8.387	4.846	11.928	0.000
	-3.263	-5.403	-1.122	0.003

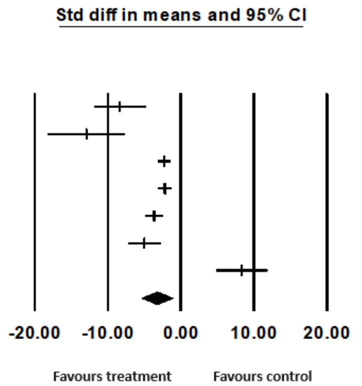

Figure 4. Forest plot of IL-6 cytokine meta-analysis results

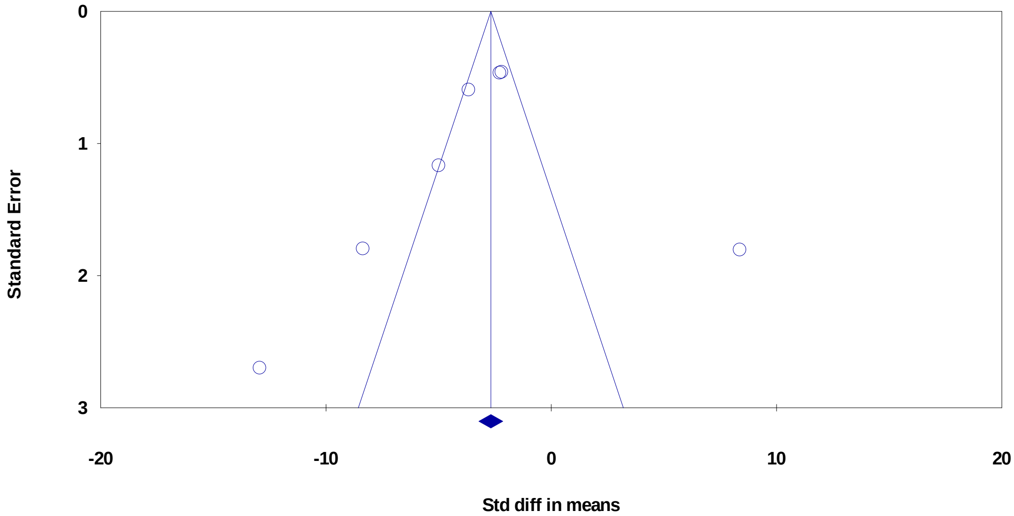

Figure 5. Funnel plot of the effect of *Phyllanthus niruri* on IL-6

Effect of *Phyllanthus niruri* on IL-6

There are 4 articles analyzing the effect of *Phyllanthus niruri* on IL-6, namely research by Giribabu et al. (2017), Ramadhani et al. (2021), Zarzour et al. (2018), and Ezzat et al. (2020). The value of variation between studies (I^2) obtained was 91.457%, reinforced by a p-value = 0.000. This shows that there is high heterogeneity between studies, so the analysis model used is the random effect model. The results of data analysis shown in the forest plot (Figure 4) show that there is a significant relationship between *Phyllanthus niruri* and IL-6 with a value of p <0.05, namely p = 0.003 and a standardized mean difference value of -3.263 (95% CI -5.403, - 1.222). This shows that the *Phyllanthus niruri* intervention reduced the inflammatory cytokine IL-6 by 3,263 times compared to the control.

The funnel plot shows a symmetrical study distribution, where the research distribution is balanced between the right and left of the midline boundary (Figure 5). This shows that there is no indeed publication bias in this meta-analysis used. The publication bias test using Egger's regression test also represented that there was no publication bias, with a P> 0.05, namely 0.322 and 0.645.

Overall, the findings of data analysis in this research indicated the same findings, namely the effect of *Phyllanthus niruri* intervention on reducing inflammatory cytokines. This can be seen from the results of the forest plot where the distribution of articles is to the left of the number 0 (line no effect). The result of the effect size is negative, indicating that *Phyllanthus niruri* can effectively reduce of the cytokines TNF-α and IL-6 during inflammation. The results of this meta-analysis support the existing theory, namely *Phyllanthus niruri* can be used as an anti-inflammatory by reducing cytokines such as TNF-α and IL-6.

Inflammation is the inherent immunological response to potentially dangerous stimuli, such as infections, damage, and metabolic stress (Antonelli & Kushner, 2017). Cell damage, which can be caused by infectious and non-infectious agent that activates inflammatory cells and sets off inflammatory signaling pathways, most frequently the NF-B, MAPK, and JAK-STAT pathways (Chen et al., 2018). Tissue immune cells like macrophages and dendritic cells release cytokines like IL-1, IL-6, and TNF- in response to foreign or self-antigens (Milenkovic et al., 2019). TNF- and IL-6 in particular play a crucial role in liver injury by sustaining hepatic inflammation (Rostami et al., 2022). The expression of IL-6, TNF-, IFN-, and IL-1 generated by macrophages may be increased in the DMBA-induced breast cancer model (Ramadhani et al., 2021).

Phyllanthus niruri contains alkaloids, flavonoids, terpenoids, saponins and triterpens. In rats with carrageenan-induced inflammation, *Phyllanthus niruri* ethanolic extract possesses anti-inflammatory effects (Sutrisna et al., 2019). Flavonoids can block transcription factors or regulatory enzymes crucial for regulating inflammatory mediators (Maleki et al., 2019). Flavonoids (e.g., quercetin, genistein, hesperetin, epigallocatechin-3-gallate) can down-regulate inflammatory indicators, inhibit the expression of pro-inflammatory cytokines (IL-6, TNF-a, IL-1b, and COX-2), and prevent brain damage. The modulation of microglial cells, which is mediated by their impacts on MAPKs and NF-kB signaling pathways, is principally related to this anti-inflammatory action (Spagnuolo et al., 2018).

Flavonoids act similarly to NSAIDs, and in-silico research revealed that quercetin could partially inhibit COX-2 by binding to subunit A, which has peroxidase activity and is a source of ROS and nitric oxide (NO), reducing production levels, cytokine expression, and modulating transcription factors like NF-kB and activating protein-1 (AP-1) (Kumar et al., 2021). It is also known that triterpene in Phyllanthus compounds can inhibit COX-2, IL-6, IL-1β, and iNOS (Yu et al., 2020). According to research of Ferrante et al. (2020), *Phyllanthus niruri* aqueous extracts have anti-inflammatory activity by lowering levels of PGE2 and 8-iso-PGF 2ά, because catechins and epicatechins inhibit COX-2 in prostate cells after lipopolysaccharide-induced mice, catechin substances were found to have anti-inflammatory effects on the iNOS protein targets.

Some of the typical lignans found in *Phyllanthus* species are phyllanthin, hypophyllanthin, and niranthin. The extract reduced COX-2 expression and the production of inflammatory mediators like TNF, PGE2, and IL-1. Furthermore, the activation of upstream signaling molecules (TLR4 and MyD88) that were essential for the activation of inflammatory signaling pathways (MAPKs, NF-B, and PI3K/Akt) was downregulated by these lignans. the conclusion of this research that phyllanthin, hypophyllanthin, and niranthin reduced the expression of MAPKs, NF-B, and Akt, which in turn lowered the expression of TNF, COX-2, and IL-1 in human macrophages (Harikrishnan et al., 2018)

Mostofa et al. (2017) found that *Phyllanthus niruri*'s methanol extract inhibited the production and release of prostaglandins, proteases, and lysosomal enzymes, which in turn reduced the infiltration of inflammatory cells. Higher doses produce effects comparable to those of the reference medication, ibuprofen. The corilagin ingredient in *Phyllanthus niruri* has been shown in studies to have the ability to block the NF-kB pathway, limit the release of TNF-a, IL-1b, and IL-6 cytokines, and increase nitric oxide production, both of which are inflammatory mediators, in rats with acute cholestatic circumstances (Jia et al., 2013).

The important findings of this study are scientific evidence and provide a significant theoretical basis for the potential of *Phyllanthus niruri* as an anti-inflammatory. These results strengthen the scientific basis for the use of *Phyllanthus niruri* as a supplement for the community to prevent inflammation

CONCLUSION

Phyllanthus niruri affected the reduction of inflammatory cytokines with an effect size of SMD on TNF-α is -17,611 (95% CI -29,347, -5,875; p=0.003) and IL-6 is -3,263 (95% CI -5,403, -1,222; p = 0.003). The results of this meta-analysis strengthen the concept/theory that *Phyllanthus niruri* has the potential as an anti-inflammatory by reducing the inflammatory cytokines TNF-α and IL-6. Further research is needed to focus on the differences in potential of certain parts (leaves, stems, flowers) of *Phyllanthus niruri* as an anti-inflammatory.

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