

# Fractionation, Phytochemical Profiling, and Antioxidant-Antimicrobial Activities of *Tadehagi triquetrum* L. Leaves

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**Abstract.** *Tadehagi triquetrum* L., locally known as Duduk leaves, has long been used in traditional medicine for various treatments, including treating fever, liver disorders, and kidney ailments. However, systematic phytochemical and bioactivity studies on this species remain scarce. Unlike previous reports that mainly focus on crude extracts, this study integrates polarity-based fractionation, quantitative phytochemical analysis, antioxidant evaluation, antimicrobial testing, and Liquid Chromatography–Mass Spectrometry (LC-MS) profiling to provide a more comprehensive assessment of the bioactive potential of *T. triquetrum* L. leaves. The crude extract obtained from maceration with 70% ethanol yielded 28.49%. Subsequent fractionation using n-hexane, chloroform, ethyl acetate, and water produced yields of 6.52%, 0.90%, 12.82%, and 52.37%, respectively. Phytochemical screening revealed that the ethyl acetate fraction contained the highest levels of phenolics, tannins, and flavonoids. This fraction exhibited the strongest antioxidant activity in the DPPH assay ( $IC_{50} = 0.71 \mu\text{g/mL}$ ). LC–MS profiling identified 15 putative compounds, with catechin (26.84%) and kaempferol (14.58%) as the dominant constituents. The ethyl acetate fraction also demonstrated moderate to strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. These findings highlight *T. triquetrum* L. as a promising source of natural antioxidant and antimicrobial agents.

## 1 Introduction

Indonesia, a country with exceptionally high biodiversity, has a long-standing tradition of utilizing medicinal plants for various health treatments. The accessibility of herbal medicines in both rural and urban areas contributes to their continued popularity, as they are generally perceived as affordable and culturally familiar treatment options [1]. In recent decades, growing concerns about the adverse effects and resistance associated with synthetic pharmaceuticals have renewed global interest in discovering natural products with potent pharmacological activities. Among these, plants rich in secondary metabolites such as flavonoids, alkaloids, tannins, and phenolics are recognized as promising candidates for the development of antioxidant and antimicrobial agents [2].

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**Fig 1.** *Tadehagi triquetrum* (L.) Leaves

*Tadehagi triquetrum* (L.), commonly known as “duduk leaves” in Indonesia, belongs to the Fabaceae family and is traditionally used to treat fever, inflammation, kidney and liver disorders, and urinary problems, as well as to stimulate appetite and relieve pain. This shrub, which can grow up to three meters in height, is widely distributed across tropical regions from India to Southeast Asia, including Java Island, reflecting its ecological adaptability and abundance [2].

Ethanollic extracts often contain impurities such as lipids and pigments that can interfere with Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. To minimize these interferences and concentrate bioactive constituents, solvent fractionation based on polarity is commonly employed. Fractionation is essential for differentiating bioactive constituents in medicinal plants, as crude extracts contain chemically complex mixtures that can mask structure–activity relationships. Polarity-guided fractionation selectively enriches specific compound classes, enabling clearer correlation between chemical composition and biological activity. Semi-polar fractions, such as ethyl acetate, are frequently enriched with phenolics and flavonoids and often exhibit stronger antioxidant and antimicrobial activities [3]. Previous studies on other plant species, such as *Ficus platyphylla*, have demonstrated that polar fractions facilitate more effective compound identification than crude extracts by enhancing the detection of minor metabolites and improving analytical precision [4]. Despite its ethnomedicinal relevance, scientific investigations on *T. triquetrum* L. remain limited compared with other members of the *Desmodieae* tribe. *T. triquetrum* is taxonomically related to the *Desmodium* genus, whose species have been extensively studied for their diverse bioactivities and phenolic-rich compositions [5]. Although *T. triquetrum* L. is phylogenetically related to these well-studied taxa, its chemical composition and bioactivity profile at the fraction level have not been adequately explored. In particular, comprehensive investigations integrating polarity-based fractionation, quantitative phytochemical profiling, and correlation with antioxidant and antimicrobial activities remain scarce. Therefore, this study aims to perform solvent-based fractionation, phytochemical screening, LC–MS compound profiling, and evaluation of antioxidant and antimicrobial activities of *T. triquetrum* L. leaf extracts. The results are expected to provide scientific validation of the traditional uses of *T. triquetrum* L. and to enhance understanding of its phytochemical and biological characteristics.

## 2 Materials and Methods

### 2.1 Materials

Dried *T. triquetrum* L. leaves were collected from Tawangmangu district, 70% Ethanol (analytical grade), *n*-hexane, chloroform, ethyl acetate, and distilled water were used as solvents. All reagents for phytochemical tests were of analytical grade.

### 2.2 Extraction of *Tadehagi triquetrum* L.

A total of 1000 g of dried powder leaves of *T. triquetrum* L. were macerated in 6000 mL of 70% ethanol (1:6 w/v) for 72 hours, with occasional stirring every 24 hours. The solvent was replaced eight times to ensure exhaustive extraction. The combined filtrates were concentrated under reduced pressure at 40-65°C using a rotary evaporator until a constant weight was obtained, yielding the ethanolic crude extract. The extraction yield was calculated based on the ratio of the extract mass to the initial dry plant material.

### 2.3 Fractionation

Sequential solvent fractionation was performed using solvents of increasing polarity: *n*-hexane, chloroform, ethyl acetate, and water. Twenty grams of the ethanolic extract were suspended in 50 mL of distilled water and partitioned successively with an equal volume of each solvent in a separatory funnel (1:1 v/v). Each solvent fraction was gently shaken for approximately 5 minutes, allowed to separate, and then concentrated under reduced pressure using a rotary evaporator. The obtained fractions were weighed, and their yields were determined gravimetrically.

### 2.4 Phytochemical Screening

The crude extract and solvent fractions were subjected to standard phytochemical tests to identify major classes of secondary metabolites [6]. Alkaloids were detected using Dragendorff's, Mayer's, and Wagner's reagents; saponins were confirmed by the formation of persistent froth; tannins were indicated by a dark green or blue coloration upon reaction with FeCl<sub>3</sub>; flavonoids were identified by the appearance of a red to orange color after treatment with magnesium and hydrochloric acid; and steroids or terpenoids were confirmed by characteristic color changes in the Liebermann–Burchard reaction.

### 2.5 Quantitative Phytochemical Analysis

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method with gallic acid as the standard, and the results were expressed as mg gallic acid equivalent (GAE)/g extract. Total tannin content (TTC) was measured following a similar procedure, using tannic acid as the reference standard. The total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method with quercetin as the standard, and results were expressed as mg quercetin equivalent (QE)/g extract. All absorbance readings were recorded using a visible spectrophotometer (Thermo Scientific Genesys 20), and compound concentrations were calculated from linear regression equations derived from calibration curves.

## 2.6 Antioxidant Activity

The antioxidant activity of the crude and fractionated extracts was evaluated using the DPPH free radical scavenging assay. A 30 µg/mL ethanolic DPPH solution served as the control. Stock solutions of each sample (1000 ppm) were serially diluted to obtain various concentrations: 1.953–500 ppm for the ethanolic extract, chloroform, and aqueous fractions, and 1–10 ppm for the *n*-hexane and ethyl acetate fractions. Equal volumes (1 mL) of DPPH solution and sample were mixed and incubated in the dark for 60 minutes. The absorbance was then measured at 517 nm. Ascorbic acid (2–4 ppm) was used as a positive control. The percentage of DPPH radical scavenging activity was calculated, and IC<sub>50</sub> values were determined from the linear regression between percentage inhibition and sample concentration.

## 2.7 LC-MS Analysis

The fraction exhibiting the highest antioxidant activity was subjected to LC–MS (Thermo Fisher Scientific) equipped with an Ultra High Performance Liquid Chromatography (UHPLC) system coupled to a Q Exactive Orbitrap mass spectrometer to identify its bioactive components. Approximately 0.5 g of the dried fraction was dissolved in ethyl acetate and filtered before injection into the LC–MS system. Compound separation was achieved on a Hypersil Gold C18 column (150 mm × 2.1 mm; particle size 3 µm). Mass spectrometric detection was carried out using Electrospray Ionization (ESI) operated in both positive and negative ion modes, depending on the ionization properties of the analytes. Data were processed using MestReNova or MassLynx software, and compound identification was achieved by comparing the obtained spectra with reference data from the MassBank database.

## 2.8 Antimicrobial Activity

The fraction showing the highest antioxidant activity was further evaluated for antimicrobial potential against *Staphylococcus aureus* and *Escherichia coli* using the disk diffusion method. Sterile paper disks impregnated with the test samples were placed on agar plates previously inoculated with the test microorganisms. After 24 h incubation at 37 °C, the diameters of inhibition zones were measured to assess antibacterial efficacy. The chloramphenicol was used as a positive control, and Aquadest as a negative control.

# 2 Results and Discussion

## 3.1 Extraction and Fractionation

The extraction of *T. triquetrum* L. leaves was carried out using 70% ethanol by maceration. Ethanol was chosen as the solvent due to its intermediate polarity, low toxicity, and broad solvation capacity for both polar and moderately nonpolar phytochemicals. The procedure yielded 284.91 g of crude extract from 1000 g of dried leaf powder, corresponding to a 28.49% yield. The crude extract was subsequently partitioned by liquid–liquid fractionation using solvents of increasing polarity, *n*-hexane, chloroform, ethyl acetate, and water, to facilitate the separation of compounds based on their polarity differences. The respective fraction yields were 6.52% (*n*-hexane), 0.90% (chloroform), 12.82% (ethyl acetate), and 52.37% (aqueous). Among these, the aqueous fraction exhibited the highest recovery, suggesting that polar constituents such as phenolics, flavonoids, glycosides, and tannins

predominated in the crude extract. The ethyl acetate fraction, which displayed a moderate yield, was expected to contain semi-polar compounds.

### 3.2 Phytochemical Screening

Phytochemical screening of the ethanolic extract and its solvent fractions is shown in Table 1. The data revealed the presence of alkaloids, saponins, tannins, flavonoids, and terpenoids, while steroids were absent. The ethanolic extract exhibited the most comprehensive profile, indicating that 70% ethanol efficiently extracted both polar and semi-polar compounds from *T. triquetrum* L. leaves. Alkaloids were detected in the ethanolic extract, n-hexane, and aqueous fractions using Dragendorff's, Mayer's, and Wagner's reagents, confirming the formation of alkaloid–metal complexes.

Tannins and flavonoids were consistently detected in all samples, reflecting their abundance and polar nature, while terpenoids were found mainly in the ethanolic, n-hexane, and ethyl acetate fractions, suggesting their semi-polar to non-polar characteristics. The dominance of these bioactive groups, particularly flavonoids, tannins, and alkaloids, supports the reported antioxidant and antimicrobial potential of *T. triquetrum* L. The differential distribution of compounds across fractions of varying polarity highlights the crucial role of solvent selection in isolating target metabolites and provides a chemical basis for the plant's traditional medicinal applications.

**Table 1.** Phytochemical Screening Data

Phytochemical test	Ethanolic extract	n-hexane fraction	Chloroform fraction	Ethyl Acetate fraction	Aqueous fraction
Alkaloid <i>Dragendorff</i>	+	+	-	-	+
Alkaloid <i>Mayer</i>	+	+	-	-	-
Alkaloid <i>Wagner</i>	+	+	+	+	+
Saponin	+	+	-	-	+
Tannin	+	+	+	+	+
Flavonoid	+	+	+	+	+
Terpenoid	+	-	-	+	-
Steroid	-	-	-	-	-

Information: detected (+), not detected (-)

### 3.3 Quantitative Phytochemical Content

#### 3.3.1 Total Phenolic Content (TPC)

The total phenolic content (TPC), determined by the Folin–Ciocalteu method and expressed as mg gallic acid equivalent per gram (mg GAE/g), ranged from 18.94 to 50.29 mg GAE/g. The ethyl acetate fraction showed the highest phenolic concentration (50.29 mg GAE/g), followed by the ethanolic extract (44.16 mg GAE/g), n-hexane (34.42 mg GAE/g), aqueous (28.00 mg GAE/g), and chloroform (18.94 mg GAE/g) fractions. The predominance of phenolics in the ethyl acetate fraction indicates that this medium-polarity solvent efficiently extracts phenolic compounds while minimizing the co-extraction of non-phenolic materials such as carbohydrates and proteins.

### 3.3.2 Total Tannin Content (TTC)

Tannin content, expressed as mg tannic acid equivalent per gram (mg TAE/g), ranged between 44.80 and 121.95 mg TAE/g. The ethyl acetate fraction exhibited the highest tannin concentration (121.95 mg TAE/g), followed by the ethanolic extract (106.87 mg TAE/g), *n*-hexane (82.90 mg TAE/g), aqueous (67.11 mg TAE/g), and chloroform (44.80 mg TAE/g) fractions. This distribution aligns with the qualitative screening results, indicating that ethyl acetate has a strong affinity for hydrolyzable tannins and phenolic polymers, which are moderately polar in nature.

### 3.3.3 Total Flavonoid Content (TFC)

The total flavonoid content (TFC), measured as mg quercetin equivalent per gram (mg QE/g), varied from 53.53 to 78.19 mg QE/g. The ethyl acetate fraction again displayed the highest flavonoid concentration (78.19 mg QE/g), followed by the ethanolic crude extract (71.54 mg QE/g), *n*-hexane (66.00 mg QE/g), chloroform (59.90 mg QE/g), and aqueous fraction (53.53 mg QE/g). The higher flavonoid concentration in the ethyl acetate fraction can be attributed to the solvent's semi-polar nature, which allows selective extraction of flavonoid compounds while minimizing co-extraction of non-phenolic substances such as sugars, proteins, chlorophyll, and resins. These findings suggest that flavonoids, along with phenolics and tannins, constitute the major antioxidant components responsible for the biological activity of the plant.

## 3.4 Antioxidant Activity

The antioxidant activity of the crude ethanolic extract and its solvent fractions was evaluated using the DPPH radical scavenging assay, expressed as IC<sub>50</sub> values (the concentration required to inhibit 50% of DPPH radicals) [7]. Ascorbic acid serves as a positive control. The results in Table 2, demonstrated a wide range of activities, with IC<sub>50</sub> values varying from 0.71 to 29.27 µg/mL. The ethyl acetate fraction exhibited the strongest radical scavenging activity (IC<sub>50</sub> = 0.71 µg/mL), comparable to the ascorbic acid standard (IC<sub>50</sub> = 0.46 µg/mL), followed by the ethanolic extract (1.23 µg/mL), aqueous fraction (2.15 µg/mL), *n*-hexane (2.70 µg/mL), and chloroform fraction (29.27 µg/mL).

**Table 2.** IC<sub>50</sub> Values of Ethanolic Extract, Fractions, and Ascorbic Acid

Sample	IC <sub>50</sub> (µg.mL <sup>-1</sup> )	Antioxidant Strength
Ethanolic extract	1.23	Very strong
<i>n</i> -hexane fraction	2.70	Very strong
Chloroform fraction	29.27	Very strong
Ethyl acetate fraction	0.71	Very strong
Aqueous fraction	2.15	Very strong
Ascorbic acid (standard)	0.46	Very strong

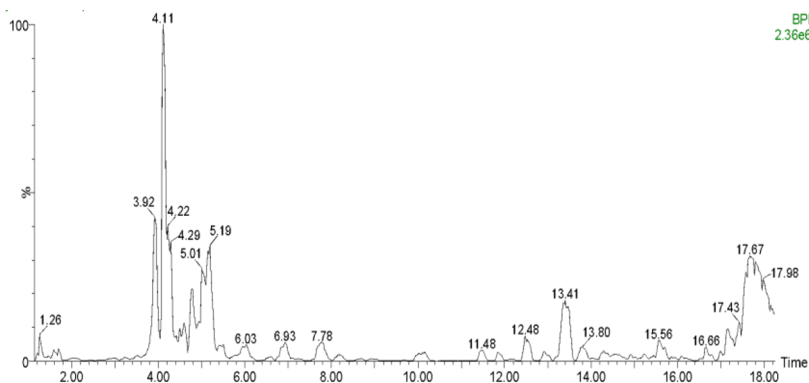
Based on the classification criteria for antioxidant strength (IC<sub>50</sub> < 50 µg/mL), all extracts and fractions of *T. triquetrum* L. exhibited very strong antioxidant activity. The consistent decrease in IC<sub>50</sub> values with increasing TPC, TFC, and TTC indicates a strong correlation between antioxidant performance and the abundance of these compounds. These findings are consistent with previous reports emphasizing that phenolics and flavonoids play a pivotal role in scavenging free radicals through electron or hydrogen donation mechanisms. In contrast, a previous study on another family, the antioxidant profile of *Desmodium triquetrum* that was observed in the methanolic extract exhibited an IC<sub>50</sub> value of 82.66 µg/ml

[7]. The remarkable activity of the ethyl acetate fraction suggests that medium-polar solvents are most effective in concentrating semi-polar antioxidant constituents such as flavonoids, phenolic acids, and tannins. These metabolites can donate electrons or hydrogen atoms to neutralize DPPH radicals, thereby interrupting oxidative chain reactions and stabilizing reactive species [8].

In comparison to the crude extract, the fractionation process significantly enhanced antioxidant efficiency by concentrating active molecules and reducing interfering substances. This finding aligns with previous reports on *Ficus platyphylla* and *Desmodium gangeticum*, where ethyl acetate fractions also exhibited the most potent antioxidant properties [4-5]. Therefore, the high antioxidant activity of *T. triquetrum* L.'s ethyl acetate fraction provides scientific support for its traditional use as a natural antioxidant source for pharmacological or nutraceutical applications.

### 3.5 LC-MS Analysis of the Ethyl Acetate Fraction

The ethyl acetate fraction, which exhibited the strongest antioxidant activity, was further analyzed using LC-MS to identify its bioactive constituents. The chromatogram revealed 15 prominent peaks with distinct retention times, corresponding to compounds tentatively identified through comparison with reference spectra from the MassBank database. The chromatographic profile and suspected compounds are shown in Figure 2 and Tabel 3. Notably, the comprehensive LC-MS profiling of the ethyl acetate fraction of *T. triquetrum* L. leaves remains scarce in the literature, and the present study provides one of the first detailed chemical fingerprints linking semi-polar secondary metabolites to antioxidant and antimicrobial bioactivities in this species. Compared to prior reports on *T. triquetrum* L. or closely related taxa, which primarily focus on crude extracts or total phenolic content.



**Fig. 2.** LC-MS Chromatogram of Ethyl Acetate Fraction

The LC-MS results showed that catechin (26.84%), and kaempferol (14.58%) were identified as the putative dominant components, based on their relative peak areas. It should be noted that these compound identifications are tentative, as they are based on LC-MS spectral data and database comparisons. Therefore, further confirmatory analyses such as co-injection with authentic standards, high-resolution MS/MS, and/or NMR spectroscopy are recommended for unequivocal structural confirmation. The dominance of catechin and kaempferol suggests that flavonoid-driven redox modulation plays a central role in the observed antioxidant activity of the ethyl acetate fraction, particularly through mechanisms involving direct radical scavenging and metal ion chelation.

**Table 3.** Suspected Active Compounds in Ethyl Acetate Fraction from LC-MS analysis

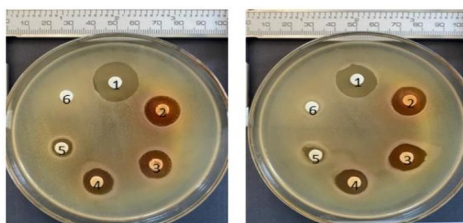
No.	Retention Time	% Area	Molar Mass	Compound	Group
1	1.26	1.20	217.07	2,3-dimethyl-5-nitro-1,7-ethanoindole	Indole alkaloids
2	1.71	0.97	268.10	Asperphenamate	Non-ribosomal peptides
3	3.92	0.03	579.15	Procyanidine	Flavonoids
4	4.11	26.84	291.08	Catechin	Flavonoids
5	5.19	14.58	287.05	Kaempferol	Flavonoids
6	6.04	1.36	577.13	Xanthomegnin	Polyketides
7	6.92	1.52	433.11	Coumestan base + 2O, O-Hex	Flavonoids
8	7.78	1.80	287.05	Kaempferol	Flavonoids
9	10.07	1.02	343.29	7-hydroxy-pregna-1,4-dien-3-one-20-carbaldehyde	Steroids
10	11.48	0.67	371.32	Aspidocarpine	Alkaloids
11	12.48	1.64	304.30	Cetylpyridinium	Quaternary ammonium
12	13.41	5.95	372.34	Methyl 1,2,2,6,6-pentamethyl-4-piperidyl sebacate	Alkaloids
13	13.80	0.85	332.33	Sanguinarine	Benzophenanthridine alkaloids
14	15.56	1.73	354.34	Proadifen	Aromatic amines
15	16.66	0.73	607.29	Reserpine	Indole alkaloids

These compounds are known for their significant biological activities, particularly antioxidant and antimicrobial properties. Catechin, a well-known flavonoid, demonstrated the second-largest peak area. This compound plays a crucial role as a natural antioxidant, containing two benzene rings along with a dihydrocarbon to which the hydroxyl group is attached to carbon 3. Moreover, catechin exerts antibacterial effects by damaging bacterial cell membranes and disrupting DNA synthesis pathways. Catechin also has a long history of use in the treatment of heart disease [9]. Kaempferol, another flavonoid detected in significant quantities, is recognized for its antioxidant, antimicrobial, anticancer, and antiviral activities. It scavenges free radicals and stabilizes reactive oxygen intermediates, thus preventing oxidative stress-induced cellular injury [10]. The predominance of flavonoid and alkaloid compounds in the ethyl acetate fraction supports the high antioxidant activity observed in the DPPH assay. Collectively, the LC-MS results confirm that the ethyl acetate fraction of *T. triquetrum* L. leaves is enriched with bioactive secondary metabolites that synergistically contribute to its potent antioxidant activities.

### 3.6 Antimicrobial Activity

The antimicrobial assay was performed on the ethyl acetate fraction of *T. triquetrum* L. leaves, which exhibited the highest antioxidant activity, to assess the correlation between antioxidant potential and antibacterial effects. The disc diffusion method was used to test *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) at concentrations of 100, 300, 500, and 700 mg/mL. Chloramphenicol (30 µg/disc) and distilled water served as positive and negative controls, respectively. Based on the Clinical and Laboratory Standards Institute (CLSI, 2020) criteria, inhibition zones were categorized as susceptible ( $\geq 18$  mm), intermediate (13–17 mm), or resistant ( $\leq 12$  mm) [11]. The inhibition zones obtained for *S. aureus* and *E. coli* at different concentrations are presented in Figures 3-4 and Tables 4-5.

The ethyl acetate fraction demonstrated inhibitory activity against both bacteria, with the largest inhibition zones observed at 700 mg/mL concentration,  $9.88 \pm 0.21$  mm for *S. aureus* (moderate but classified as resistant according to CLSI) and  $13.33 \pm 1.37$  mm for *E. coli* (strong, intermediate category). These results indicate that *E. coli* exhibited higher sensitivity to the fraction than *S. aureus*, likely due to differences in cell wall structure and permeability. While *S. aureus* (Gram-positive) possesses a thick peptidoglycan layer that limits compound diffusion, the outer membrane of *E. coli* (Gram-negative) may facilitate better interaction with semi-polar bioactive molecules [12]. These results suggest that the antimicrobial activity of *T. triquetrum* L. is primarily driven by the action of flavonoids, as the major component identified by LC-MS. Flavonoid compounds can denature bacterial proteins and damage cytoplasmic membranes, leading to leakage of cellular contents.



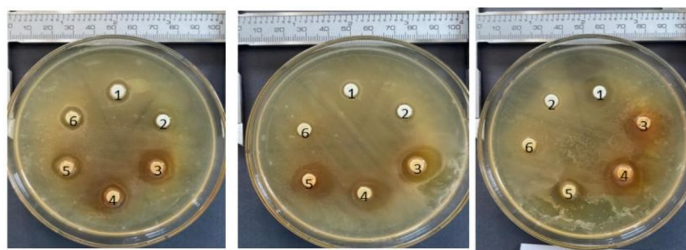
**Fig. 3.** Antimicrobial Activity of Ethyl Acetate Fraction of *T. triquetrum* L. Leaves against *S. aureus*. (1) Control (+), (2) 700 mg/mL, (3) 500 mg/mL, (4) 300 mg/mL, (5) 100 mg/mL, and 6. Control (-)

**Table 4.** Antimicrobial Activity of Ethyl Acetate Fraction of *T. triquetrum* L. Leaves against *S. aureus*.

Concentration (mg/mL)	Diameter of Inhibition Zone (mm)			Average Diameter (mm)	Category	CLSI Category
	I	II	III			
100	3.96	3.33	1.96	$3.08 \pm 1.02$	Weak	Resistant
300	8.10	8.56	8.45	$8.37 \pm 0.24$	Moderate	Resistant
500	9.40	9.44	9.58	$9.47 \pm 0.09$	Moderate	Resistant
700	10.08	9.88	9.67	$9.88 \pm 0.21$	Moderate	Resistant
Chloramphenicol (+)	14.01	14.63	11.75	$13.46 \pm 0.52$	Strong	Intermediate
Aquadest (-)	0.00	0.00	0.00	0.00	-	-

**Table 5.** Antimicrobial Activity of Ethyl Acetate Fraction of *T. triquetrum* L. Leaves against *E. coli*

Concentration (mg/mL)	Diameter of Inhibition Zone (mm)			Average Diameter (mm)	Category	CLSI Category
	I	II	III			
100	4.76	2.88	3.04	$3.56 \pm 1.04$	Weak	Resistant
300	7.24	12.36	7.73	$9.11 \pm 2.83$	Moderate	Resistant
500	9.94	11.91	12.74	$11.53 \pm 1.44$	Strong	Resistant
700	11.75	14.17	14.08	$13.33 \pm 1.37$	Strong	Intermediate
Chloramphenicol (+)	14.49	13.41	11.59	$13.16 \pm 4.44$	Strong	Intermediate
Aquadest (-)	0.00	0.00	0.00	0.00	-	-



**Fig. 4.** Antimicrobial Activity of Ethyl Acetate Fraction of *T. triquetrum* L. Leaves against *E. coli*. (1) Control (+), (2) 700 mg/mL, (3) 500 mg/mL, (4) 300 mg/mL, (5) 100 mg/mL, and 6. Control (-)

## 4 Conclusion

Extraction of *T. triquetrum* L. (duduk) leaves using 70% ethanol yielded 28.49% of crude extract, which, upon solvent fractionation, produced n-hexane (6.52%), chloroform (0.90%), ethyl acetate (12.82%), and aqueous (52.37%) fractions. Quantitative phytochemical analysis revealed that the ethyl acetate fraction contained the highest levels of total phenolics, tannins, and flavonoids, supported by LC–MS identification of catechin (26.84%) and kaempferol (14.58%) as the major putative constituents. This fraction exhibited the strongest antioxidant activity ( $IC_{50} = 0.710 \mu\text{g/mL}$ ), indicating a very strong radical scavenging potential consistent with its high polyphenolic content. In antimicrobial assays, the ethyl acetate fraction showed moderate inhibition against *S. aureus* and strong inhibition against *E. coli*, suggesting selective antibacterial efficacy. Overall, the results demonstrate that the ethyl acetate fraction of *T. triquetrum* L. leaves is a rich source of phenolic-based bioactive compounds with potent antioxidant and moderate antimicrobial properties, providing scientific support for its traditional medicinal use and potential application as a natural antioxidant or antimicrobial agent.

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