Trop J Nat Prod Res, May 2025; 9(5): 1958 - 1963 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical Constituents, Antioxidant and Anti-inflammatory Potentials of Dechlorophyllized Extract of Kenikir Leaves (*Cosmos caudatus* Kunth)

Zainal Abidin*, Aminah, Rais Razak, Mamat Pratama

Pharmaceutical Chemistry Division, Faculty of Pharmacy, Universitas Muslim Indonesia-Makassar, Indonesia

ARTICLE INFO

Article history:
Received 16 January 2025
Revised 25 March 2025
Accepted 02 April 2025
Published online 01 June 2025

ABSTRACT

Phytochemicals are a group of natural compounds or secondary metabolites derived from plants. These secondary metabolites exert various pharmacological effects, including antioxidant and anti-inflammatory properties. Several studies have shown that *Cosmos caudatus* Kunth contains phenolic compounds, flavonoids, tannins, and saponins. Various pharmacological effects, including antibacterial, antifungal, antioxidant, anti-inflammatory, antiosteoporosis, antihypertensive, and antidiabetic properties of this plant have been investigated. This study aimed to determine the total phenolic, flavonoid, tannin, and saponins contents, as well as evaluate the antioxidant and anti-inflammatory activities of the dechlorophyllized extract of *Cosmos caudatus* Kunth leaves. The crude and dechlorophyllized extracts of *Cosmos caudatus* Kunth leaves were obtained by maceration in ethanol, and liquid-liquid extraction of the crude ethanol extract, respectively. The total phenolic, flavonoid, tannin, and saponins contents of the dechlorophyllized extract were determined following standard methods. The antioxidant activity was determined using the ferric reducing antioxidant power (FRAP) and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. The anti-inflammatory activity was evaluated using the protein denaturation inhibition assay. The results showed that the total phenolic, flavonoid, tannin, and saponins contents of the dechlorophyllized extract of *Cosmos caudatus* Kunth leaves were 52.537 mgGAE/g, 47.215 mgQE/g, 47.263 mgTAE/g, and 188.177 mgSE/g extract, respectively. The dechlorophyllized extract exhibited strong antioxidant activity with FRAP value of 14.028 mg/LQE, and IC_{50} of 17.77 ppm in the DPPH radical scavenging activity. The dechlorophyllized extract also exhibited potent anti-inflammatory activity with IC_{50} value of 113.400 ppm. These findings revealed *Cosmos caudatus* Kunth leaves as a potential source of antioxidant and anti-inflammatory agents.

Keyword: Dechlorophyllation, Kenikir plant, *Cosmos caudatus*, Antioxidant, Secondary Metabolites.

Introduction

Chemical compounds in plants do not only come from the products of primary metabolic processes, but also from secondary metabolic processes commonly called secondary metabolites. Secondary metabolites are not involved in important processes in plants, but have been found to have great pharmaceutical and pharmacological benefits. [1] Various medicinal products from plants, that are used directly or indirectly as medicines have an important role in modern medicine. [2] One of the plants that has been used in traditional medicine is *Cosmos caudatus* Kunth. (Kenikir plant), where fresh leaves and leaf extracts have been used for various therapeutic purposes. [3]

The leaves of *Cosmos caudatus* Kunth have a unique smell and aroma, and as such are used as flavoring ingredients in food, and can also be eaten raw. The plant has been shown to possess numerous pharmacological effects, including antibacterial, antifungal, antioxidant, anti-inflammatory, antiosteoporosis, antihypertensive, and antidiabetic effects. [3 4]

*Corresponding author. E-mail: zainal.abidin@umi.ac.id
Tel.: +6282338805287

Citation: Abidin Z, Aminah, Razak R, Pratama M. Phytochemical Constituents, Antioxidant and Anti-inflammatory Potentials of Dechlorophyllized Extract of Kenikir Leaves (*Cosmos caudatus* Kunth). Trop J Nat Prod Res. 2025; 9(5): 1958 – 1963 https://doi.org/10.26538/tjnpr/v9i5.11

Phytochemical studies have shown that *Cosmos caudatus* Kunth. contains phenols, flavonoids, tannins, and saponins, [5] and several studies have reported the antioxidants and anti-inflammatory effects of these chemical compounds.[5] [6]In the present study, the phytochemical constituents, and pharmacological activity such as the antioxidant and anti-inflammatory activities of the Dechlorophyllized extract of the leaves of *Cosmos caudatus* Kunth were investigated. Determining the best extraction method for plant samples is the initial stage in phytochemical studies, and plays an important role in determining the types and amount of the chemical compounds extracted. The results of the extraction process are in the form of crude extracts that contain a complex mixture of chemical compounds.[7 2]

Crude extracts normally contain pigments such as chlorophyll, and this can affect the quality of the extract obtained, therefore, the chlorophyll pigment can be removed through dechlorophyllation process.[8] Dechlorophyllation of the extract reduces the chlorophyll content of the leaf extract, so that the active substances in the extract becomes more concentrated, and expected to have better biological activity. [9]

Materials and Methods

Chemicals, reagents, and equipment

The solvents and other chemicals used were of analytical grade and were obtained from commercial suppliers. Ethanol, n-hexane, ethyl acetate, sodium carbonate, methanol, bovine serum albumin (BSA), tris buffer saline (TBS), Folin-Ciocalteau reagent, aluminium chloride, hydrochloric acid, potassium acetate, trichloroacetic acid, ferric chloride, phosphate buffer, potassium ferricyanide, acetic acid, and perchloric acid

were products of Merck-Germany. Gallic acid and tannic acid (Sigma Aldrich-China), quersetin (Sigma Aldrich-Japan), vanillin (Trade Mark TCI-Tokyo), sapogenin and 2, 2-diphenyl-1-picrylhydrazyl (Sigma Aldrich-Germany). All glassware were product of Pyrex. The instruments used were UV-Vis spectrophotometer (Genesis 10S UV-Vis-Thermo Scientific) and Rotary Vacuum Evaporator (IKA RV-10 digital).

Plant collection and identification

Fresh leaves (Figure 1) of *Cosmos caudatus* Kunth. were obtained from Barru Regency, South Sulawesi, Indonesia (GIS coordinates: -4.406147, 119.603119) in November 2023. The plant was identified and authenticated at the Plant Unit of Laboratory of Pharmacognocy and Phytochemistry, Faculty of Pharmacy, Universitas Muslim Indonesia with voucher specimen number: 0084.

Extraction of plant material

The leaves of *Cosmos caudatus* Kunth. were cleaned with running water, after which they were shade-dried, and then pulverized. The powdered leaves (100 g) leaves were extracted with 200 mL ethanol (96%) by maceration at room temperature 72 hours. The extract was filtered, and it was re-maceration many times until the extraction was exhaustive. The combined ethanol extract was evaporated using a rotary evaporator at 40ºC under reduced pressure to obtain a crude ethanol extract.

Dechlorophyllation of the crude ethanol extract

The crude ethanol extract was dechlorophylated by liquid-liquid extraction (LLE). Briefly, 4 g of the crude ethanol extract was dissolved in 20 mL of methanol and partitioned with 20 mL n-hexane in 250 mL separatory funnel. The hexane layer was removed, and the process was repeated until the n-hexane layer became clear. The methanol layer was collected and evaporated in a rotary evaporator to obtain dried Dechlorophyllized methanol extract, which was subsequently weighed, and stored in an air-tight container until needed. [10]

Determination of percentage chlorophyll removal

The percentage chlorophyll removal was determined using UV-Vis Spectrophotometer. The absorbance's of the crude ethanol extract and the Dechlorophyllized extract were recorded at 660 nm. Percentage chlorophyll removal was calculated using the following equation:

$$\text{Chlorophyll removal (\%)} = \frac{\text{Abs b} - \text{Abs a}}{\text{Abs b}} \text{ x } 100\%$$

Where Abs b is the absorbance of the crude ethanol extract, and Abs a is the absorbance of the Dechlorophyllized extract. [11]

Determination of total phenolic content

The total phenolic content of the Dechlorophyllized extract was determined using the modified Folin-Ciocalteu method. The Dechlorophyllized extract (1 mL of 300 mg/L) in ethanol was mixed with 1 mL of Folin Ciocalteau reagent (10%). The mixture was allowed to stand at room temperature for 5 minutes, followed by the addition of 1 mL of 7% sodium carbonate. The mixture was incubated at room temperature for 30 minutes, thereafter, the absorbance was measured at 754 nm using a UV-Visible spectrophotometer. Gallic acid (8, 12, 16, 20, and 24 mg/L) was used to prepare a standard calibration curve. The determination was performed in triplicate and the total phenolic content of the extract was determined as mg gallic acid equivalent per gram of extract (mg GAE/g extract). [12]

Determination of total flavonoid content

The total flavonoid content of the Dechlorophyllized extract was determined using the aluminium chloride colorimetric method with minor modification.[13] Briefly, 1 mL of the Dechlorophyllized extract (500 mg/L) in ethanol was added to 1 mL of 2% aluminium chloride and 1 mL of 120 mM potassium acetate. The mixture was incubated at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 431 nm using a UV-Visible spectrophotometer. A standard calibration curve of quercetin (20, 30, 40, and 50 mg/L) was prepared from which the total flavonoid content was determined. The determination was done in triplicate, and the total flavonoid content was reported as mg quercetin equivalent per gram extract (mg QE/g extract).

Determination of total tannin content

The total tannin content of the Dechlorophyllized was determined according to the method previously described by Ojha *et al*. [14] with minor modification. Aliquot (0.5 mL) of 500 mg/L of the Dechlorophyllized extract in ethanol was mixed with 8 mL of distilled water, 0.5 mL of 0.1 M ferric chloride and 0.5 mL of 8 mM potassium ferricyanide were added, and the mixture was incubated at room temperature for 10 minutes. The absorbance of the reaction mixture was measured at 756 nm using a UV-Vis spectrophotometry. A calibration curve of tannic acid (15, 20, 25, 30, and 35 mg/L) was used as standard. The determination was done in triplicate, and the total tannin content of the extract was reported as mg tannic acid equivalent per gram extract (mg TAE/g extract).

Determination of total saponins content

The total saponins content of the Dechlorophyllized extract was estimated according to vanillin-acetic acid method with minor modifications. Briefly, 0.6 mL of 500 mg/L extract in ethanol, was evaporated to dryness. To the dried extract was added 0.2 mL of freshly prepared 5% vanilin-acetic acid solution, and 0.8 mL of perchloric acid. The mixture was incubated at 60ºC for 15 minutes, after which the reaction mixture was cooled in an ice bath for 20 second, followed by the addition of 5 mL of glacial acetic acid. The absorbance of the mixture was measured at 466 nm using a UV-Visible spectrophotometer. A standard calibration curve of sapogenin (60, 80, 100, 120, and 140 mg/L) was prepared. The determination was performed in triplicate, and the total saponins content was reported as mg sapogenin equivalent per gram extract (mg SE/g extract). [15, 16]

Determination of antioxidant activity

Reducing power assay

Antioxidant activity was determined using Ferric Reducing Antioxidant Power (FRAP) method with some modification. The extract solution (1 mL of 1000 mg/L) in ethanol was mixed with 1 mL 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide, then incubated at 50ºC for 20 minutes. After the incubation period, the reaction was stopped by the addition of 1 mL of 10% trichloroacetic acid, then centrifuged at 3000 rpm for 10 minutes. To the supernatant (1 mL) was added 1 mL of distilled water, and 0.5 mL $FeCl_3$ (0.1%). The mixture was incubated at room temperature for 5 minutes. The absorbance of the mixture was measured at 720 nm using a UV-Visible spectrophotometer. A standard calibration curve of quercetin (10, 15, 20, 25, and 30 mg/L) was prepared. The experiment was done in triplicate. Increasing absorbance of the mixture indicates increased reducing power. The antioxidant activity in terms of the reducing power was determined as mg/L of quercetin equivalent (mg/LQE). [17]

DPPH radical scavenging assay

Antioxidant activity was determined using the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging method. Briefly, 1 mL of various concentration of the extract (5, 10, 15, 20, and 25 mg/L) was mixed with 3 mL of 35 mg/L DPPH solution in methanol. The mixture was incubated at room temperature in the dark for 30 minutes. The absorbance of the reaction mixture was measured at 517 nm using a UV-Visible spectrophotometer. The antioxidant activity was calculated as percentage inhibition of free radical using the following equation : [18 19]

$$\% \text{ Inhibition} = [(A_{Control} - A_{Extract})/A_{Control}] \text{ x } 100\%$$

Where $A_{Control}$ is the absorbance of DPPH solution without the extract and $A_{Extract}$ is the absorbance of the extract in DPPH solution. The half maximal inhibitory concentration (IC_{50}) was calculated from a regression analysis. Lower IC_{50} value indicates higher antioxidant activity.

Determination of anti-inflammatory activity

Protein albumin denaturation assay

Anti-inflammatory activity was determined using protein albumin denaturation assay as described by Almira *et al*. [20] with some modification. A 100 µL of various concentration of the extract (50, 150, 250, 350 mg/L) was mixed with 0.2% bovine serum albumin (BSA) in Tris-buffered saline (pH 6.2) up to 5 mL total volume. The mixture was incubated at room temperature for 25 minutes, then heated at 90ºC for 5 minutes. The mixture was allowed to cool at room temperature,

thereafter, the absorbance was measured at 660 nm, using ethanol in 0.2% BSA as a negative control. The anti-inflammatory activity was calculated using the following equation. [20]

$$\text{Inhibition (\%)} = [(A_{Control} - A_{Extract})/A_{Control}] \times 100\%$$

Where $A_{Control}$ is the absorbance of negative control and $A_{Extract}$ is the absorbance of the extract in BSA. Percentage inhibition greater than 20% indicates anti-inflammatory activity.

Results and Discussion

Extraction and Yields of crude and dechlorophyllized extracts of Cosmos caudatus leaves

The yields of the crude and dechlorophyllized extracts of *Cosmos caudatus* leaves are presented in Table 1. The results showed a moderate yield (8.24%) for the crude extract, and high yield (36.75%) for the dechlorophyllized extract. This observation is consistent with the results from some previous studies, which showed that the leaf part of plants contain higher phytoconstituents than other parts of plant. [21 - 23] Previous studies also showed that ethanol extract gives higher yield than other solvent extracts.[24, 25] In the present study, crude ethanol extract was obtained by maceration of the leaves of *Cosmos caudatus* Kunth. with ethanol. The maceration method is a method suitable for the extraction of thermolabile chemical compounds.[2] On the other hand, dechlorophyllized extract was obtained by liquid-liquid extraction (LLE) method. In this partition process, two immiscible solvents; methanol and n-hexane were used. Chlorophyl in the crude ethanol extract has higher solubility in n-hexane than in methanol, so chlorophyll is partitioned into the hexane layer, resulting in the dechlorophyllization of the crude extract. This happens because nonpolar solvents are more effective in removing chlorophyll than polar solvents. Chlorophyll beside containing hydrophilic head of porphyrin ring with a central magnesium atom, contains a long lypophyllic hydrocarbon tail, making it more soluble in nonpolar solvents. [26]

The use of n-hexane as a nonpolar solvent in LLE method is expected to achieve dechlorophyllization of the crude extract without affecting the biological activity of the extract, and in some cases increase the biological activity of the extract,[9,10] unlike other nonpolar solvents such as chloroform and petroleum ether which in addition to achieving dechlorophyllization, would result in a decreased biological activity of the extract. [26]

The efficiency of the dechlorophyllization process (percentage chlorophyll removal) was determined spectrophotometrically at 660 nm. A higher absorbance value of the crude ethanol extract compared to the dechlorophyllized extract indicates the removal of chlorophyll. The result of the chlorophyll removal is presented in Table 2.

Figure 1: *Cosmos caudatus* Kunth. leaves

Phytochemical content of dechlorophyllized extract of Cosmos caudatus leaves

Total phenolic content

Phenolic compounds have antioxidant activity that play a role in the prevention and treatment of many chronic diseases such as diabetes, liver damage, inflammation, cancer, cardiovascular diseases, neurological disorders and many others. Within the structure of phenolic compounds, there are conjugated double-bonds and chromophoric groups that enable their determination by UV-Visible spectrophotometry.[27] The determination of total phenolic content was carried out using the Folin-Ciocalteau method. This method is based on the oxidation-reduction reaction that occurs between the hydroxyl groups in the phenolic compound and the Folin-Ciocalteau reagent to create a blue molybdenum-tungsten complex. The intensity of the blue colour is proportional to the concentration of phenolic compounds, so the higher the concentration of phenolic compounds that will reduce heteropoly acids (phospholipids-phosphotungstats) to molybdenum-tungsten complexes, the more intense is the colour produced. [28]

The total phenolic content was estimated from the calibration curve of the standard solution of gallic acid. The calibration curve produced a linear correlation coefficient (r) of 0.9987 and a regression equation y = 00203x + 00446. The total phenolic content of the dechlorophyllized ethanol extract of *Cosmos caudatus* leaves was obtained as 52.537 mg GAE/g extract (Table 3).

Table 1: Yields of crude and dechlorophyllized extracts of *Cosmos caudatus* Kunth leaves

Sample	Weight of leaves (g)	Weight of crude extract (g)	Extraction yield (g)	Percentage yield (%)
Crude extract	100.08	-	8.25	8.24
Dechlorophyllized extract	-	4.00	1.47	36.75

Table 2: Percentage chlorophyll removal of the dechlorophyllization process

Replicate	Absorbance B	Absorbance A	Chlorophyll removal level (%)	Average level (%)
1	0.331	0.196	40.785	
2	0.286	0.188	34.265	42.282±8.86
3	0.334	0.161	51.796	

Table 3: Total phenolic content of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth. leaves

Replicate	Absorbance	Total phenolic content (mg GAE/g extract)	Average (mg GAE/g extract)
1	0.354	48.335	
2	0.382	53.732	52.537±3.75
3	0.390	55.545	

Total flavonoid content

Flavonoids are polyphenol derived compounds that consist of several subclasses specifically flavonols, chalcones, isoflavones, flavones, and flavanones. Flavonoids have been shown to possess numerous biological activities, such as anticancer, antiangiogenic, anti-inflammatory, antioxidant, antiallergic, and antimicrobial activities. [29]

The determination of flavonoid content of dechlorophyllized extract of *Cosmos caudatus* leaves was carried out using aluminium chloride ($AlCl_3$) and potassium acetate (CH_3COOK) to produce a basic environment. The mechanism involved in the determination is based on the reaction of $AlCl_3$ with flavonoid compounds in a basic environment to create aluminum-flavonoid complex characterized by yellow coloured solution. [13]

The total flavonoid content was estimated from a calibration curve of standard solution of quercetin. The calibration curve was linear with correlation coefficient (r) of 0.9989 and a regression equation of y = 0.0325x – 0.4149. The total flavonoid content of the dechlorophyllized ethanol extract of *Cosmos caudatus* leaves was obtained as 47.215 mg QE/g extract (Table 4).

Table 4: Total flavonoid content of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth. Leaves

Replicate	Absorbance	Total flavonoid content (mg QE/g extract)	Average (mg QE/g extract)
1	0.352	47.194	
2	0.330	45.840	47.215±1.38
3	0.375	48.610	

Table 5: Total tannin content of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth. leaves

Replicate	Absorbance	Tannin content (mg TAE/g extract)	Average (mg TAE/g extract)
1	0.520	44.801	
2	0.523	46.394	47.263±2.99
3	0.574	50.594	

Table 6: Total saponins content of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth. leaves

Replicate	Absorbance	Saponin content (mg SE/g extract)	Average (mg SE/g extract)
1	0.423	181.447	
2	0.427	182.971	188.177±10.36
3	0.472	200.114	

Table 7: Antioxidant activity (Ferric reducing antioxidant power) of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth. leaves

Replicate	Absorbance	Antioxidant activity (mg/LQE)	Average Antioxidant activity (mg/LQE)
1	0.575	13.145	
2	0.584	13.914	14.028±0.94
3	0.597	15.025	

Table 8: Antioxidant activity (DPPH radical scavenging activity) of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth. leaves

Concentration (mg/L)	Absorbance	Absorbance DPPH	% Inhibition	IC_{50} (mg/L)
5	0.526	0.716	26.536	
10	0.454	0.716	36.592	
15	0.407	0.716	43.156	17.17
20	0.325	0.716	54.608	
25	0.261	0.716	63.547	

Total tannin content

Tannins are secondary metabolites that exist in plants and are known to have many therapeutic benefits. Plant extracts containing tannins can be used as diuretics, as astringents, to treat diarrhea, gastric tumors, duodenal tumors, as antibacterial, anti-inflammatory, antiseptic, antiviral, as well as hemostatic agents. [30] Total tannin content of the extract was determined using ferric chloride ($FeCl_3$) and potassium ferricyanide ($K_3[Fe(CN)_6]$) reagents.[14] The determination is based on the complexation of $FeCl_3$ and potassium ferricyanide with tannins to produce a bluish-green solution. The intensity of the coloured complex is proportional to the concentration of tannins, and this can be measured spectrophotometrically.

The total tannin content was estimated from a calibration curve of a standard solution of tannic acid. The calibration curve was linear with correlation coefficient (r) 0.9955 and a regression equation y = 0.0213x - 0.019. The total tannin content of the dechlorophyllized ethanol extract of *Cosmos caudatus* leaves was obtained as 47.263 mg QE/g extract (Table 5).

Total saponins content

Saponins are natural glycoside detergents that have amphiphilic surface activity and have a large molecular weight. Saponins are divided into two groups, namely; steroidal saponins and triterpenoidal saponins. Saponins are known to have antimicrobial properties, and protect plants from insect attacks, lower serum cholesterol level, have antioxidants, antivirals, anti-inflammatory, antibacterial, antiparasitic, antitumor and anticarcinogenic properties. [16 31]

The determination of saponins levels was carried out using a vanillin-acetic acid reagent with perchloric acid. The principle of the determination is based on the oxidation reaction of saponins with vanillin, using perchloric acid as an oxidizer, resulting in the formation of a purple coloured solution. [16]

The total saponins content was estimated from a calibration curve of a standard solution of sapogenin. The calibration curve produced a linear relationship with correlation coefficient (r) of 0.9947 and a regression equation of y = 0.005x – 0.0533. The total saponins content of the dechlorophyllized ethanol extract of *Cosmos caudatus* leaves was obtained as 188.177 mg SE/g extract (Table 6).

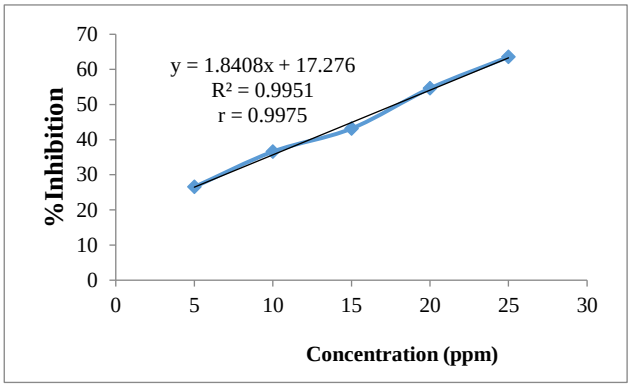

Figure 2: Concentration-response curve of DPPH radical scavenging activity of dechlorophyllized ethanol extract of *Cosmoscaudatus* Kunth. leaves

Pharmacological activity of dechlorophyllized ethanol extract of Cosmos caudatus leaves

Antioxidant activity

Ferric reducing antioxidant power (FRAP)

Antioxidants are substances that delay, prevent or eliminate oxidative damage to a target molecule and inhibitors of oxidation processes, even at relatively small concentrations, thus they play significant physiological roles in the body. One method of measuring antioxidant activity is the Ferric Reducing Antioxidant Power (FRAP) method. This technique assesses the total antioxidant content of an ingredient by evaluating the ability of an antioxidant compounds to reduce ferric (Fe^{3+}) ion to ferrous (Fe^{2+}) ion. Thus, the antioxidant power of a compound is directly related to its capacity to reduce ferric ions. [32]

The determination of antioxidant activity of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth leaves was achieved through a calibration curve of quercetin standard solution. The calibration curve yielded a linear relationship with correlation coefficient (r) of 0.9987, and a regression equation of y = 0.0117x – 0.4212. The ferric reducing antioxidant power of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth leaves was obtained as 14.028 mg/LQE (Table 7).

DPPH radical scavenging activity

Antioxidants are compounds that can slow down the oxidation process of free radicals. One of the mechanisms of action of antioxidant compounds is by hydrogen atom or proton donation to radical compounds to compensate for the lack of electrons by free radicals, and in the process

inhibit the chain reaction of free radical formation by stabilizing more radical compounds. Antioxidants in the extract neutralize DPPH radicals by providing electrons to DPPH, resulting in a change in colour from purple to yellow or reduced the intensity of the purple colour. The antioxidant activity is measured in terms of IC_{50} value, which is the concentration of the extract required to neutralize 50% of DPPH radicals. The smaller the IC_{50} value, the stronger the antioxidant activity. [19]
The results of the DPPH radical scavenging activity of dechlorophyllized ethanol extract of *Cosmos caudatus leaves is presented* in Table 8 and Figure 2.

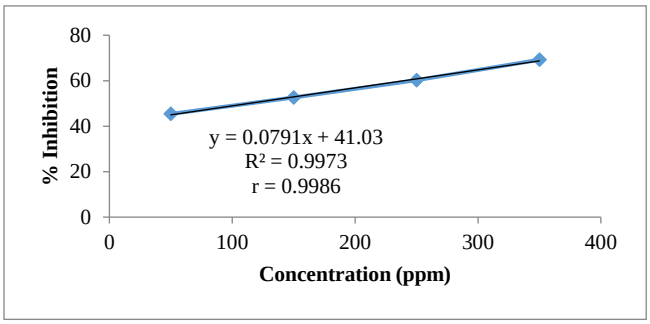

Figure 3: Concentration-response curve of anti-inflammatory activity of dechlorophyllized ethanol extract of *Cosmoscaudatus* Kunth. leaves

Table 9: Anti-inflammatory activity of dechlorophyllized ethanol extract of *Cosmoscaudatus* Kunth. Leaves

Concentration	Absorbance	% Inhibition	IC_{50} (mg/L)
Negative control	1.335	-	
50	0.729	45.39	
150	0.633	52.58	113.400
250	0.531	60.22	
350	0.411	69.21	

The linear regression equation of the concentration-response curve of DPPH scavenging activity of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth leaves was y = 1.8408x + 17.276, while the correlation coefficient (r) of 0.997, and coefficient of determination (r^2) of 0.9951 were obtained. Based on the IC_{50} value of 17.77 mg/L (<50 mg/L), the dechlorophyllized ethanol extract of the leaves of *Cosmos caudatus* Kunth. was categorized as having very strong antioxidant activity.

Antiinflammatory activity
The anti-inflammatory activity of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth leaves was evaluated by the protein denaturation inhibition method as an initial screening test for anti-inflammatory activity. Protein denaturation is a process in which proteins lose their tertiary and secondary structures due to the effect of external factors, such as strong acids, strong bases, concentrated organic salts, organic solvents, and heat. Protein denaturation can be a cause of inflammation. Compounds that can inhibit protein denaturation are potential anti-inflammatory drugs. [33]
The anti-inflammatory activity of dechlorophyllized ethanol extract of the leaves of *Cosmos caudatus* Kunth was estimated from a calibration curve of the sample solution based on its absorbance value. The absorbance of the sample solution was measured at a wavelength of 660 nm. The calibration curve as shown in Figure 3 produced a linear relationship with correlation coefficient (r) of 0.9973 and the regression equation y = 0.0791x – 41.03. The result of the anti-inflammatory activity of dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth leaves was reported as IC_{50} value, and the IC_{50} value of 113.400 ppm was obtained (Table 9).
The percentage of protein denaturation (IC) in the dechlorophyllized ethanol leaf extract of *Cosmos caudatus* Kunth increased in line with increasing concentration. The protein denaturation inhibition percentage range from 45.39 to 69.21%. According to Almira *et.al*., compounds that inhibit protein denaturation greater than 20% are considered to have anti-inflammatory properties.[20]

Conclusion

The results from the present study revealed high contents of phenols, flavonoids, tannins, and saponins in the dechlorophyllized ethanol extract of *Cosmos caudatus* Kunth leaves. The dechlorophyllized extract of *Cosmos caudatus* Kunth leaves exhibited strong antioxidant activity and potent anti-inflammatory activity. These findings revealed that *Cosmos caudatus* Kunth leaves have the potential as a source of antioxidant and anti-inflammatory agents. Therefore, further research should be conducted to test other activities, in the form of antidiabetes and anti-tyrosinase.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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