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Original Research Article

Optimisation of Phenolic Content and Antibacterial Activity of *Cosmos caudatus* Kunth. Leaf Ethanol Extract Using different Drying Techniques

Yuri P. Utami[1]*, Imrawati Imrawati[2], Ismail Ismail[3], Suwahyuni Mus[4], Ainun Jariah[5], Rahmah Mustarin[5], Wa O. Dytha Amalia Lestari M[6], Safira Waliulu[6]

[1*]*Department of Pharmaceutical Biology, Faculty of Health Sciences, University of Almarisah Madani, South Sulawesi, Indonesia.*
[2]*Department of Pharmaceutical and Chemical Analysis, Faculty of Health Sciences, University of Almarisah Madani, Indonesia.*
[3]*Department of Pharmacy and Pharmaceutical Technology, Faculty of Health Sciences, University of Almarisah Madani, Indonesia.*
[4] *Department of Clinical Pharmaceutical, Faculty of Health Sciences, University of Almarisah Madani, Indonesia.*
[5]*Program of Pharmacy Studies, Faculty of Medicine and Health Sciences of Muhammadiyah University of Makassar, South Sulawesi, Indonesia.*
[6]*Bachelor of Pharmacy, Faculty of Health Sciences, University of Almarisah Madani, South Sulawesi, Indonesia.*

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ABSTRACT

Cosmos caudatus Kunth. leaves contain alkaloids, flavonoids, tannins, saponins, and steroids that can act as antibacterials. Drying methods can affect the chemical content and antibacterial potential of plant leaves. This study aims to optimise the chemical content and the antibacterial activity of *Cosmos caudatus* leaves against *Staphylococcus aureus* and *Escherichia coli* using different drying techniques. The leaves were dried using four methods: direct sun, indirect sun (under shade), oven 40 ℃, and air-dried. The extracts were then obtained by maceration using 70% ethanol. The phenolic contents were tested spectrophotometrically. The antibacterial activity was tested against two bacteria strains using the Kirby-Bauer test (disc paper) with different extract concentrations (5%, 10%, and 15%). The antibacterial activity was computed from the zones of inhibition. The study's results showed the total phenolic content of *C. caudatus* Kunth. leaf extract by direct sunlight was 1.90 ± 0.06, indirect sunlight = 2.50 ± 0.02, air-dried = 2.51 ± 0.41, and oven (40°C) dried = 1.85 ± 1.01. Extracts from the different techniques produce different inhibitory zones against the bacteria strains. The direct sunlight-drying method has a greater inhibitory effect on *S. aureus* than *E. coli*, compared to indirect sun-drying, oven and air-drying techniques. This suggests that different drying techniques influence the total phenolic content and antibacterial potential of ethanol leaf extracts of *Cosmos caudatus*.

***Keywords*:** *Cosmos caudatus* Kunth, drying, *Staphylococcus aureus*, *Escherichia coli*, Kirby-Bauer test, phenolic.

Introduction

Antibacterial compounds can interfere with bacterial growth or metabolism. Based on their toxicity, antibacterials exhibit bactericidal and bacteriostatic (bacterial growth inhibition) activities. Bacteriostatic agents only inhibit bacterial growth, while bactericidal agents kill bacteria. However, bacteriostatics can be bacteriocidal at high concentrations.[1]

Infectious diseases are the most common in some developing countries, including Indonesia. Infection is when microorganisms (bacteria, viruses and fungi) enter the body, multiply and cause disease.[2] Pathogenic bacteria (*E. coli* and *S. aureus*) have been implicated in various infections in humans and livestock, including digestive tract diseases, diarrhoea, and skin infections.[3] *E. coli* bacteria are normal in the intestines, while *S. aureus* bacteria are normal flora bacteria on human skin and mucous membranes. However, if the growth of the bacteria is abnormal or excessive, it becomes pathogenic and causes various diseases.[4]

*Corresponding author. E mail: yuriutami88@gmail.com
Tel: +6285395374908

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The leaves of *C. caudatus* Kunth are one of the naturally occurring plant components that exhibit antibacterial properties. Preliminary studies on the phytochemistry of *C. caudatus* leaves, extracted with ethanol and other solvents, show the presence of flavonoids, saponins, terpenoids, alkaloids, tannins, and essential oils that have antimicrobial potential.[5] The flavonoid compounds exhibit antibacterial potential by causing damage to bacterial cell membranes, followed by the release of intracellular chemicals and complex molecules linked to proteins that impede the function of cell membranes.[6] It is believed that tannins can cause cell membranes or walls to shrink, thereby disrupting cell permeability, slowing the growth of the walls between them, or making the cell membrane unable to carry out living activities.[6] Alkaloids in leaf extract interfere with the composition of peptidoglycans through a reaction between the base group of the alkaloid and the amino acid compounds that make up the cell wall, which are not integrally formed.[7] Saponins act as an antibacterial by destroying cell wall permeability, leading to cell death.[8]

Several antibacterial activity test studies have been carried out on *C. caudatus*. Leaves extracted with 96% ethanol have the best antibacterial activity, with an inhibitory zone of 4.7 mm against *S. aureus*.[5] Moreover, n-hexane, ethyl acetate, water fractions, and ethanol extract obtained from *C. caudatus* leaves at 10%, 15%, 20%, 25%, and 30% inhibited bacillary dysentery bacteria. Specifically, the microorganisms *Shigella boydii* ATCC 12985, *Shigella flexneri* ATCC 12022, and *Shigella dysenriae* ATCC 13313 are susceptible to leaf extracts of *C. caudatus*.[9]

The content of active plant compounds can be influenced qualitatively and quantitatively by drying methods and conditions (temperature).[10] The use of drying methods at different temperatures affects the total

levels of compounds, namely phenolics, in plants. Research has shown that the highest total phenols, flavonoids, and tannins are obtained from drying natural ingredients.[11] It has been shown that phenolic compounds possess antibacterial activity.[12]
This study aimed to optimise the total phenolic content and antibacterial activity against *S. aureus* and *E. coli* of the ethanol extract of *C. caudatus* using different drying techniques.

Materials and Methods

Sampling
The leaves of *C. caudatus* used for this study were obtained from the village of Leang-Leang Kec. Bantimurung Kab.Maros (Figure 1), South Sulawesi Province (4°57'24 "S 119°42'19" E), on January 12, 2023. The leaf sample of *C. caudatus* Kunth was identified at the Plant Determination Unit of the Pharmacognosi-Phytochemistry, Faculty of Pharmacy laboratory of the Muslim University of Indonesia (Voucher No. 0059/C/UD-FF/UMI/IX/2023).
Fresh leaf samples were collected, cleaned, washed with running water, cut out of the fresh samples and subjected to different drying processes. Four drying techniques were employed: direct sunlight (DS), indirect sunlight (IS), air-dried (AD), and oven (40°C). Drying was done until the water level reached <10%. The dried sample was ground using a blender and then covered with mesh 40. After that, the samples were subjected to further drying using four drying techniques: direct sunlight (DS) for 14 hours, indirect Sunlight (IS) for 56 hours, air-dried (AD) for 144 hours, and using an oven at a temperature of 40 °C for 8 hours.[13]

Determination of Water Content
Toluene distillation was used to determine the water content. The toluene used was saturated with water first. The sample (5 g) was transfered into a round bottom flask, and the saturated toluene was added. The flask was heated for 15 minutes. Once the toluene begins to boil, the process is stopped, and the solvent is allowed to drop off at a drip rate of 2 drops/second, which is increased to 4 drops/sec. After all the water has drained, the sample is further heated for another 5 minutes. The sample is cooled to room temperature. The amount of water is measured using the equation below after the toluene and water have completely separated.[14]

$$\% \text{ Water Content} = \frac{Water\ Volume}{Sampel\ Weight}\ x\ 100\%$$

Extraction
The powdered plant sample (230 g) was macerated with 70% ethanol at 1:5 (sample to solvent) for 3x24 hours with occasional stirring. The extract was stopped on the 3rd day and then filtered. The marc was macerated again using the same solvent as before. The combined filtrates were concentrated at 40℃, and the yield obtained was calculated from the equation below.

$$Yield\ (\%) = \frac{Extract\ weight}{Dry\ Simplicia\ Weight}\ X\ 100\ \%$$

Phytochemical screening testing using TLC
a. Alkaloids
The extract was weighed and dissolved in 70% ethanol. The solution was applied to the TLC plate using a capillary tube and eluted with chloroform and ethyl acetate (7:3) as the mobile phase with 3 drops of acetic acid. After that, the plate was dried and sprayed with Dragendroff's reagent. The sample is said to be positive for alkaloids if an orange or brown colour appears under a 366 nm UV lamp.[15]

b. Flavonoids
The extract was weighed and dissolved in 70% ethanol. The solution was applied to the TLC plate using a capillary tube and eluted with chloroform and ethyl acetate (7:3) as the mobile phase with 3 drops of acetic acid. After that, the plate is dried and sprayed with an aluminium chloride reagent. The sample is said to be positive for flavonoids if the stain fluoresces yellow under a 366 nm UV lamp.[15]
c. Tanins
The extract was weighed and dissolved in 70% ethanol. The solution was applied to the TLC plate using a capillary tube and eluted with chloroform and ethyl acetate (7:3) as the mobile phase with 3 drops of acetic acid. After that, the plate was dried and sprayed with a 5% $FeCl_3$ reagent. The sample was said to be positive for tannin if black spots appeared under a 366 nm UV lamp.[15]

d. Terpenoid
An amount of the extract was weighed and then dissolved in 70% ethanol. The solution was spotted onto the TLC plate using a capillary tube and eluted with chloroform and ethyl acetate. (7:3). The plate was dried and sprayed with the H_2SO_4 reagent. The plate was first heated and then observed at 366 nm UV light. The sample was said to be positive for steroids if there appeared to be a brownish-red stain.[15]

Determination of phenolic content
The sample (10 mg) was dissolved in ethanol to a concentration of 1 mg/mL, and 10 µL was taken and mixed in a well (96-well microplate) that had previously been filled with 100 µL of distilled water. About 0.4 µL of 10% Folin-Ciocalteu reagent and 25 µL of 7.5% Na_2CO_3 solution were added to each well and incubated for 30 min at room temperature. The phenolic content was determined using a microplate reader by measuring the sample absorbance at maximum wavelength. The total phenolic amount of the sample was determined using the standard phenolic acid curve with a range of concentrations of 2, 3, 4, 5, and 6 µg/mL, and the result was expressed as milligrams of gallic acid equivalent per gram of the extract (mg/g GAE).[16] Total phenolic content was calculated using the following formula:

$$Total\ phenolic\ GAE = \left(\frac{mg}{g} GAE\right) = C\left(\frac{V}{W}\right)$$

Information :
C: total phenolics from a standard curve (mg/L)
V: extract volume
W: extract weight (g)

Medium Creation
Production of Nutrient Media (NA)
The nutrient agar media using 5 g dissolved in 250 mL of distilled water and heating it on a hot plate, then sterilising it in an autoclave at a temperature of 121°C for 15 minutes.[17]

MHA Medium Spraying
MHA medium (9.5 g) was suspended in 250 mL of distilled water. The medium was heated until it boiled and adequately mixed. The pH was measured at 7.4 and transferred into tubes or bottles to be sterilised in an autoclave for 15 minutes at a temperature of 121ºC.[18]

Rejuvenation of Pure Culture
One tube of *E. coli* and *S. aureus* bacterial culture each was inoculated into the frozen NA agar slant medium separately and aseptically by placing the tube needle containing the culture on the bottom of the agar slant and pulling it using a zig-zag movement (streak method). Next, it was incubated in an incubator at 37ºC for 24 hours.[19]

Figure 1: A picture of *C. caudatus* Kunth leaves in its natural habitat (Private collection)

Bacterial Test Suspension Manufacturing
A total of 2 samples of test bacteria were rejuvenated from pure culture and suspended with 0.9% NaCl 20 mL in a sterile test tube, then homogenised and incubated for 24 hours.[20]

Bacterial Activity Testing
The Kirby-Bauer method was used to test the activity of the extract against *S. aureus* and *E. coli* using a paper disc impregnated with extract concentrations of 15%, 10%, and 5% made by dissolving the extracts in 2 mL of 10% DMSO in a vial. 10 mL of MHA medium was poured into each petri dish and allowed to solidify. A sterile cotton swab dipped into the bacterial suspension was swabbed on the MHA medium petri dish(es) until the surface was covered. A paper disc that had been soaked in varying concentrations of *C. caudatus* leaf extract, tetracycline and DMSO (10%) positive and negative controls, respectively, was placed on the medium. Then, the petri dish was incubated for 24 hours at 37°C. The zone of inhibition was measured using a calliper. The treatment was carried out for *E. coli*.[21]

Results and Discussion

This study was conducted to determine the effectiveness of the drying technique of ethanol extract of *C. caudatus* leaves on its antibacterial properties. The sample was dried to reduce the moisture content and stop enzymatic reactions. Moisture content above allowable limits can become a growth medium for mould and other microorganisms. Therefore, drying can prevent a decrease in quality and damage to the herbal material. The sample was ground to increase its surface area for easy extraction of the active components.
The results shown in Table 1 show that the water content of indirect sunlight drying samples (ISD) 4 % has the lowest percentage value, whereas that of the air-dried samples (AD) 8% has the highest percentage value. This result differs from what was previously reported, that the higher the drying temperature, the less moisture is produced. Therefore, it could be inferred that exposure time affects the amount of moisture obtained.[14] This is supported by research which states that the aeration drying technique at room temperature requires the longest drying time and shows a higher water content than other techniques. The water content obtained in each sample follows the standard requirements of ≤10% for herbal drugs.[14]
The extraction process involves maceration, which was chosen because it does not require high temperatures. The use of high temperatures causes damage to specific compounds, such as polyphenolic compounds.[22] 70% ethanol was used for sample extraction because it has the property of extracting both polar and nonpolar organic compounds in plant samples due to its ability to penetrate plants' cell walls. Ethanol is also an excellent solvent for extracting polyphenols from plants.[23] From the results of the extract process, the lowest extract percentage was obtained at 40℃ with oven drying.
Table 2 shows the percentage extract yield from variations in drying techniques and drying time. Temperature variations may have caused the differences in the yields. The results indicated that the higher the drying temperature, the lower the extract value obtained (OV 35.9 %, DS 36.33%, ISD 38.69% and AD 42.17%). According to research[9], the extract value of a product will decrease with higher drying temperature. The percentage of extract yields is proportionate to the amount of compounds present in the sample matrix. Secondary plant metabolites were identified with TLC before antibacterial activity testing. The TLC profile of the extract using chloroform and ethyl acetate (7:3) as the eluting solvent mixture showed that the ethanol leaf extract of *C. caudatus* contains flavonoids, alkaloids, tannins, and saponins (ISD, DS, OV and AD), as shown in Table 3.

Determination of phenolic content
The Follin Ciocalteau reagent, which contains a polymeric ion complex made of phosphomolybdic acid and heteropolyphosphotungstic acid, is used in the total phenolic content analysis measurement. This approach is the most often used method for determining the total phenolic content in plants since it is simpler and uses the Folin Ciocalteau reagent, which reacts with phenolic compounds to generate a solution whose absorbance can be measured.[24]
In this determination, gallic acid is added to the Na_2CO_3 solution after reacting with Folin Ciocalteau's reagent to provide a yellow colour showing the presence of phenolics. The hydroxyl group in the phenolic molecule combines with Folin-Ciocalteau's reagent during the process to generate a blue molybdenum-tungsten complex with an unknown structure. The amount of phenolic ions that form determines the intensity of the blue colour complex that is formed. The more phenolic compounds there are, the more phenolic ions will reduce phosphomolybdate phosphotungstic acid into a molybdenum-tungsten complex, resulting in a more intense colour.[24]
The standard curve for gallic acid was made at 5 different concentrations (2 ppm, 3 ppm, 4 ppm, 5 ppm, and 6 ppm) to generate a linear regression equation (y = 0.0508x + 0.2308 (r = 0.9524) with a good r-value. From the regression equation, the total phenolic content of *C. caudatus* leaf extract from the different drying techniques are as shown (Table 4): direct sunlight drying method, 1.90 mgEGA/g Extract ± 0.06; Indirect sunlight = 2.50 mgEGA/g Extract ± 0.02; Air-dried = 2.51 mgEGA/g Extract ± 0.41; and at 40 °C of oven drying = 1.85 mgEGA/g Extract ± 0.01.

Table 1: Percentage Water Content of *C.caudatus* leaves sample

No	Drying Technique	Water Content
1	ISD	4%
2	DS	6%
3	OV	6%
4	AD	8%

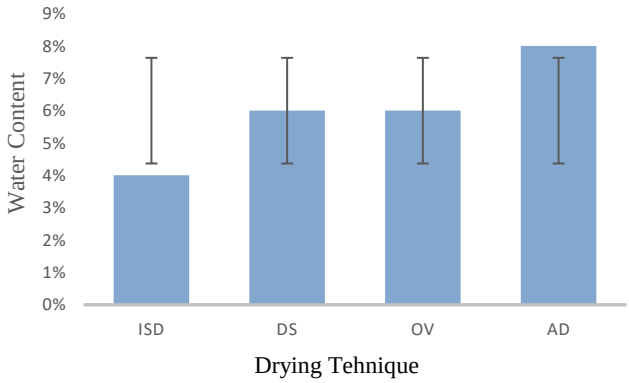

Figure 2: Results of percentage Water Content of *C.caudatus Kunth.* Leaves using different drying technique

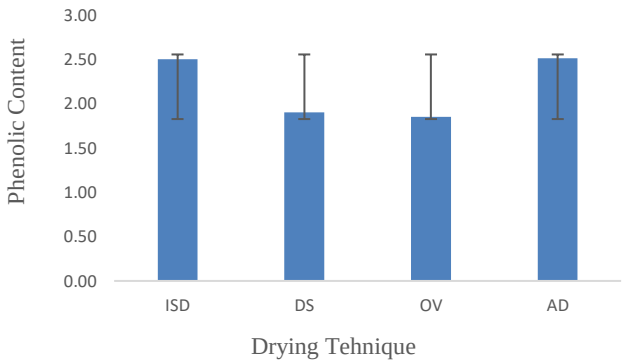

Figure 3: Phenolic Content of *C.caudatus Kunth.* leaves sample

Table 2: The percentage yield of *C. caudatus* leaf Ethanol Extract

Drying Method	Dry Sample Weight (g)	Extract Weight (g)	Yield (%)
ISD	230	89	38.69
DS	230	83.56	36.33
OV	230	82.57	35.9
AD	230	97	42.17

Table 3: Results of Qualitative Phytochemical Tests Using the TLC Method

Compound Class	Sample				Spot color
	ISD	DS	OV	AD	
Alkaloids	+	+	+	+	Orange
Flavonoids	+	+	+	+	Yellow Fluorescence
Terpenoids	+	+	+	+	Brownish Red
Tannins	+	+	+	+	Black

Table 4: Determination of Total Phenolic Content of *C. caudatus* Kunth Leaf Extract

Drying Method	Replication	Absorbance	Content Phenolics (µg/mL)	Total Phenolic Content (mg EAG/g Extract)	x ± SD
	1	0.484	4.9843	2.4921	
ISD	2	0.487	5.0433	2.5217	2.50 ± 0.02
	3	0.482	4.9449	2.4724	
	1	0.431	3.9409	1.9705	
DS	2	0.420	3.7244	1.8622	1.90 ± 0.06
	3	0.423	3.7835	1.8917	
	1	0.418	3.6850	1.8425	
OV	2	0.420	3.7244	1.8622	1.85 ± 0.01
	3	0.418	3.6850	1.8425	
	1	0.460	4.5118	2.2559	
AD	2	0.534	5.9685	2.9843	2.51 ± 0.41
	3	0.462	4.5512	2.2756	

The TPC yield is in the following order: air-dried drying technique (2.51 mgEGA/g Extract ± 0.41) > indirect sunlight drying technique (2.50 mgEGA/g Extract ± 0.02) > direct sunlight drying technique (1.90 mgEGA/g Extract ± 0.06) > 40°C oven drying (1.85 mgEGA/g Extract ± 0.01).

This result means the sample drying method can affect the total phenolic content. In this study, the air-drying method has a higher total phenolic content than other methods. This is because the phenolic compounds in the extract are easily damaged at high temperatures. That is, heating causes a decrease in the total phenolic content. Air drying sustains and increases the material's overall phenol content.[25] It protects the polyphenol components from damage due to high temperatures and thus maintains the content of phenolic compounds. It also has an impact on the total amount of phenol that can be dissolved.[26] In the early stages of the drying process, phenol compounds tend to decrease very quickly because, during drying, the phenol compounds undergo oxidation by the polyphenol oxidase enzyme to produce quinones. The polyphenol oxidase enzyme is inactivated at higher drying temperatures, which results in less enzyme activity and less phenol damage. Higher temperatures could disrupt phenol stability, so the overall quantity of phenol detected will remain constant at lower temperatures.[26]

Bacterial Activity Testing

Results from the antibacterial testing of the extract of *C. caudatus* at concentrations of 5%, 10%, and 15% against *E. coli* and *S. aureus* in the Kirby-Bauer test are shown in Tables 5-8. Results of testing antibacterial activity on *E. coli* bacteria with concentrations of 5%, 10% and 15% respectively using indirect sunlight drying (ISD), namely (9.48 mm, 9.7066 mm, and 10.91 mm), direct sunlight drying (DS), namely (8.95 mm, 9.6 mm, and 11.1966 mm), oven drying (OV) namely (9.82 mm, 10.55 mm, and 11.1066 mm) and air drying (AD) Extract namely (9.46 mm, 9.66 mm, and 10.17 mm). Meanwhile, the results of testing antibacterial activity on *S. aureus* bacteria with concentrations of 5%, 10% and 15% respectively using indirect sunlight drying (ISD), namely (9.6 mm, 10.2266 mm, and 11.3366 mm), direct sunlight drying (DS), namely (10.146 mm, 10.866 mm, and 14.7566 mm), oven drying (OV) namely (9.63 mm, 10.0833 mm, and 10.9666mm) and air drying (AD) Extract namely (10.163 mm, 10.4633 mm, and 11.6366 mm). The advantage of this method is that it is simple to carry out and can also be used to ascertain the sensitivity of various microbes to antimicrobials at specific concentrations.[5] The results indicate that, in tandem with increasing concentrations, there was a corresponding increase in the inhibition zones for the growth of *E. coli* and *S. aureus*.[27] Thus, it can be said that the larger the inhibition zone formed, the greater the effectiveness of *C. caudatus* leaf extract in inhibiting bacteria growth. Each concentration used for the antibacterial activity test has almost the same ability to inhibit *E. coli* and *S. aureus* are shown in figure 4 and 5. Based on the classification of bacterial growth inhibitory responses, the positive control showed a very potent inhibition (> 20 mm), the 15% potent inhibition (10–20 mm), the 10% concentration strong inhibition (10–20 mm), and the 5% concentration, medium inhibition category (5–10 mm). Thus, the ethanol extract of *C. caudatus* has potential as an antibacterial.[28]

The results also show that gram-positive bacteria were more susceptible to the extract from direct sunlight drying than gram-negative bacteria using indirect sun, oven, and air-drying methods. The antibacterial

activity of samples can be influenced by the class of secondary metabolites that can inhibit bacterial growth. This activity can be due to the action of individual compounds or a combination of compounds in the extract.[29] It is possible that the large zone of inhibition produced by the leaf extract occurs due to the synergy between the mechanisms of the compounds in the extract, as previously reported, that *C. caudatus* Kunth. leaf extract contains compounds with potential antibacterial properties, including phenolics such as flavonoids, tannins, and saponins.[30] It has also been reported that *C. caudatus* Kunth. leaf extract showed potent antibacterial activity against several bacterial strains.[17] Phenolics interact with proteins to generate complex molecules that impair the function of cell membranes. Intracellular chemicals are released due to the bacterial cell membrane being damaged.[31] Apart from phenolics such as flavonoids, tannins can disrupt the plasma membrane and inhibit enzyme activities[32], leading to the inhibition of bacterial metabolism, which affects bacterial growth.

Conclusion

Variations in drying techniques for *C. caudatus* leaf extract affect the phenolic content. The air-dried drying technique gave the highest total phenolic content of 2.51 mgGAE/g extract ± 0.41 compared to others. The study concludes that drying techniques affect the phytoconstituents' yield of *C. caudatus* leaf and play a significant role in its antibacterial activity.

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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Table 5: Results of the Antibacterial Activity of Indirect Sunlight Drying Extract

Microorganisms	Replication	Inhibition Zone Diameter (mm)			Control	
		5%	10%	15%	Positive	Negative
E. coli	1	10.03	10.27	11.12	20.11	5.6
	2	9.12	9.47	10.87	19.02	5.58
	3	9.29	9.38	10.74	18.7	5.63
	Average	9.48	9.7066	10.91	19.2766	5.6033
	SD	0.4838	0.4899	0.1931	0.7392	00251
S. aureus	1	9.67	10	10.62	20.42	5.66
	2	9.57	9.86	11.64	20.52	5.6
	3	9.56	10.82	11.75	20.65	5.65
	Average	9.6	10.2266	11.3366	20.53	5.6366
	SD	0.060	0.5185	0.6230	0.1153	0.0321

Table 6: Testing of antibacterial activity of extract from Direct Sunlight Drying

Microorganisms	Replication	Inhibition Zone Diameter (mm)			Control	
		5%	10%	15%	Positive	Negative
E. coli	1	9.53	10.15	11.51	20.48	5.48
	2	8.74	9.53	10.69	20.77	5.41
	3	8.6	9.12	11.39	20.2	6.13
	Rate-rate	8.9566	9.6	11.1966	20.483	5.673
	SD	0.5014	0.5185	0.4428	0.2850	0.3970
S. aureus	1	10.09	10.99	15.6	23.15	5.65
	2	10.33	11.25	15.22	20.21	5.53
	3	10.02	10.36	13.45	24.08	5.43
	Rate-rate	10.146	10.866	14.7566	22.48	5.5366
	SD	0.1625	0.4576	1.1474	2.0201	0.1101

Table 7: Testing of Antibacterial Activity Extract from Oven Drying

Microorganisms	Replication	Inhibition Zone Diameter (mm)			Control	
		5%	10%	15%	Positive	Negative
E. coli	1	9.98	10.36	10.87	24.7	6.42
	2	9.78	11.21	11.97	27.15	6.43
	3	9.71	10.09	10.48	24.4	6.35

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	Rate-rate	9.8233	10.5533	11.1066	25.4166	6.4
	SD	0.1401	0.5844	0.7726	1.5085	0.0435
S. aureus	1	9.96	10.03	10.5	24.38	5.54
	2	9.51	10.19	11.56	24.34	6.24
	3	9.42	10.03	10.84	26.48	6.56
	Rate-rate	9.63	10.0833	10.9666	25.0666	6.1133
	SD	0.2893	0.09237	0.5412	1.2241	0.5216

Table 8: Testing of Antibacterial Activity in Air Drying

Microorganisms	**Replication**	**Inhibition Zone Diameter (mm)**			**Control**	
		5%	**10%**	**15%**	**Positive**	**Negative**
E. coli	1	9.07	9.39	10.18	20.37	6.62
	2	10.16	10.26	10.31	21.42	5.72
	3	9.15	9.35	10.02	24.29	5.7
	Average	9.46	9.6666	10.17	22.0266	6.0133
	SD	0.6075	0.5142	0.1452	2.0291	0.5254
S. aureus	1	10.62	11.13	11.48	21.23	5.84
	2	9.7	9.89	11.76	21.31	5.5
	3	10.17	10.37	11.67	23.34	5.73
	Average	10.163	10.4633	11.6366	21.96	5.69
	SD	0.4600	0.6252	0.1429	1.1957	0.1734

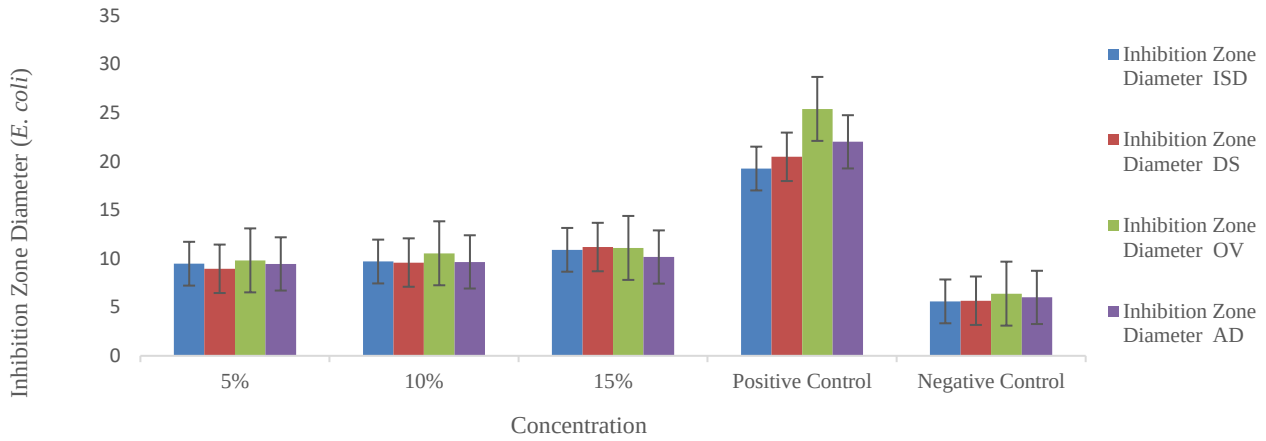

Figure 4: Antibacterial testing of the extract of *C. caudatus* against *E. coli* bacteria in the Kirby-Bauer

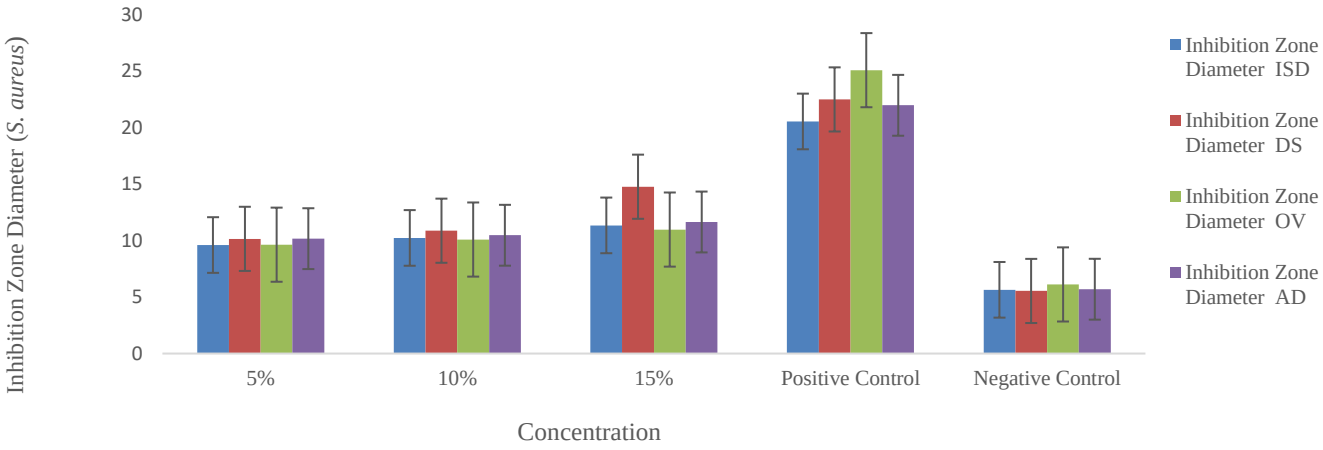

Figure 5: Antibacterial testing of the extract of *C. caudatus* against *S. aureus* bacteria in the Kirby-Bauer

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