RESEARCH PAPER

Antibacterial, Antifungal and Phytochemical Analysis of the Organic Extracts from *Hibiscus cannabinus*

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ABSTRACT

The present study aimed to investigate the phytochemical composition and selected biological activities of leaf extracts of *Hibiscus cannabinus* L. Fresh leaves were collected, dried, powdered, and extracted using four solvent systems: water, acetone, methanol, and a 1:1 mixture of acetone and methanol. The extracts were evaluated for extraction yield, total phenolic content, total flavonoid content, total tannin content, antibacterial activity, antifungal activity, and α-amylase inhibitory activity.

The phytochemical analysis revealed variations among the extracts prepared using different solvents. The 1:1 acetone–methanol extract exhibited the highest reported total phenolic content (234.9 ± 8.6), total flavonoid content (885.6 ± 24.6), and total tannin content (3981.3 ± 17.5). The α-amylase inhibitory assay we performed showed that the aqueous extract exhibited inhibition values of 12.7%, 7.8%, and 10.2% at concentrations of 1, 2, and 4 mg/mL, respectively. Overall, the study highlights the influence of solvent selection on the phytochemical composition of *H. cannabinus* leaf extracts and provides insights into their biological properties.

Keywords: *Hibiscus cannabinus* L.; kenaf; leaf extracts; solvent extraction; phytochemical analysis; total phenolic content; total flavonoid content; tannins; antibacterial activity; antifungal activity; α-amylase inhibition.

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INTRODUCTION

Hibiscus cannabinus L., commonly known as kenaf, is an annual herbaceous dicotyledonous plant belonging to the family Malvaceae. It is widely distributed in tropical and temperate regions, particularly in Asia and Africa, and is well adapted to different environmental and soil conditions. China and India are among the major producers of kenaf, together accounting for a substantial proportion of global production. The plant is capable of growing under relatively low-water conditions and can be incorporated into crop rotation systems, making it an important crop from both agricultural and environmental perspectives. The adaptability, rapid growth and multipurpose nature of *Hibiscus cannabinus* have contributed to its increasing importance as a source of agricultural, nutritional, industrial and pharmaceutical products (Sim & Nyam, 2021; Parvin et al., 2025).

Kenaf is traditionally recognised as an important fibre-producing crop, with fibres mainly obtained from the stem. Its strong and durable fibres have been used in the production of ropes, sacks, canvases, carpets and other value-added products. Kenaf fibre has also gained attention as a potential alternative to synthetic fibres because its cultivation requires comparatively fewer resources. Its applications have expanded beyond traditional fibre production, with the plant being considered a multipurpose crop with potential uses in different industries (Parvin et al., 2025).

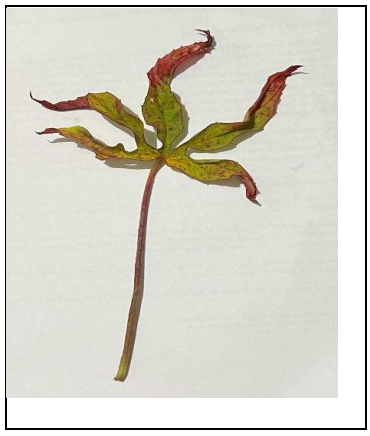


Fig 1: Morphology of *Hibiscus cannabinus*

In addition to its industrial importance, H. cannabinus has considerable nutritional and medicinal value. Different parts of the plant contain a variety of primary and secondary metabolites, including amino acids, carbohydrates, minerals, fatty acids, vitamins, dietary fibres, phenolic compounds, flavonoids, steroids and saponins. The leaves, seeds, shoots and flowers have been used as food, while kenaf seed meal and other plant materials have also been investigated for their nutritional potential in animal feed. The seeds contain oil and many nutritional components, and the seeds can be processed into flour and incorporate into food products (Sim & Nyam, 2021; Kujoana et al., 2025).

The medicinal importance of kenaf has been associated with the presence of various bioactive compounds. Leaves and seeds of H. cannabinus have been reported to possess several biological activities, including antioxidant, anticancer, analgesic, anti-inflammatory, aphrodisiac and hepatoprotective properties. The plant has also been traditionally used in different medicinal practices for the management of various disorders. Its biological properties are associated with the presence of phenylpropanoid and other phytochemical constituents. Compounds such as kaempferol, vanillic acid, syringic acid, caffeic acid, gallic acid, p-hydroxybenzoic acid, p-coumaric acid and ferulic acid have been identified from kenaf seed extracts, along with other bioactive constituents like omega-3 fatty acids and sterols (Sim & Nyam, 2021; Adnan et al., 2020).

The biological activities of kenaf are also influenced by the extraction of its phytochemical constituents. Different extraction solvents can affect the recovery of bioactive compounds and consequently influence the biological properties of kenaf extracts. Studies on the leaves and seeds of H. cannabinus have demonstrated the potential of these plant parts as sources of bioactive compounds, highlighting the importance of extraction conditions in evaluating their biological activities (Adnan et al., 2020).

The nutritional and phenolic composition of H. cannabinus further supports its potential for use in food, feed and other applications. The presence of phenolic compounds and other phytochemicals contributes to the functional value of the plant, while its nutritional constituents make it a promising resource for food and feed industries. However, the utilisation of kenaf seed-based products may be affected by the presence of certain antinutritional substances in unprocessed kenaf seeds, including tannins, oxalates, phytates, trypsin inhibitors, saponins and nitrates (Kujoana et al., 2025).

Considering the diverse phytochemical composition and reported biological properties of H. cannabinus, investigation of its organic extracts can provide important information regarding its bioactive potential. Therefore, the present study focuses on the antibacterial, antifungal and phytochemical analysis of organic extracts from *Hibiscus cannabinus L*. The study aims to evaluate the antimicrobial potential of the extracts against selected microorganisms and to identify the phytochemical constituents present in the extracts, thereby contributing to the understanding of the biological potential of this multipurpose plant.

MATERIALS AND METHODS

Fresh leaves samples of *Hibiscus cannabinus* leaves are collected from the Ema Keithel (Women's market) of Manipur, India, where the leaves are called as "Sougri mana" in their vernacular name and cultivated around the state's soil.

The preparation of the leaves for phytochemical and antimicrobial studies, begin by thoroughly washing and cleaning the leaves under tap water to remove any dirt or impurities, then patted them dry using a clean towel. Next, the cleaned leaves were spread on a tray and dried them in a hot air oven set to 60°C until they become brittle and can be easily ground into a fine powder. Once dried, the leaves are grinded into a fine powder using a mortar and pestle, then calculated the total dry weight which was 64.4g and divided it into four equal segments gaining 16.1g. Grounded Hibiscus cannabinus leaves Soxhlet extraction was then performed on each segment using different solvents: aqueous, acetone, methanol, and a 1:1 mixture of acetone and methanol, each at 20°C for 8 hours.

After extraction, the extracts were collected and dried in an oven until the liquid extracts convert to dried residues which were then collected as powder extracts form. Preparation of each dried extract by dissolving it in a solvent to achieve a concentration of 1 mg/ml, 2mg/ml and 4mg/ml. Finally, the extraction yield was calculated by comparing the initial dry weight of the powdered leaves to the final dry weight of each extracted powder.

Yield extraction calculation for Soxhlet extraction can be determined using the following formula:
Yield (%) = Weight of extracted compound / Weight of initial sample x 100

Aqueous	Acetone	Methanol	1:01 (Acetone: Methanol)
3.173g	1.775g	2.18g	2.8g

Table 1: Weight of *Hibiscus cannabinus* leaves powder used for different extraction process

Aqueous	Acetone	Methanol	1:01 (Acetone: Methanol)
19.80%	11.90%	13.60%	17.50%

Table 2: Extracted yield of *Hibiscus cannabinus* leaves with aqueous, acetone, methanol and 1:1 of acetone: methanol



Fig 2: Final extracts for Aqueous, Acetone, Methanol and 1:1 of acetone and methanol for *Hibiscus cannabinus*

Then each of the dried extracts was dissolved in specific solvents in the concentration of 1mg/ml, 2mg/ml and 4mg/ml. These solvents included pure acetone, pure methanol, and a 1:1 ratio of acetone and methanol, respectively. The following procedure was carefully followed to ensure the accurate preparation of each extract for subsequent analysis.

ANTIMICROBIAL ANALYSIS

IN-VITRO ANTIMICROBIAL ACTIVITY STUDY BY THE AGAR-WELL DIFFUSION METHOD; The antimicrobial activity of aqueous, acetone, methanol and 1:1 of acetone and methanol extracts from *Hibiscus cannabinus* leaves was evaluated against bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* and a fungus that is *Candida albicans* using the agar-well diffusion method, with slight modifications (Perez et al., 1990; Bauer et al., 1966). In brief, nutrient agar and potato dextrose agar were prepared in a clean environment under laminar airflow. Each media plate was prepared by pouring 25.0 mL of nutrient agar (NA) into a 10.0 cm glass petri dish, resulting in a uniform media thickness of approximately 4.0 mm.

ALPHA AMYLASE INHIBITORY TEST; The alpha-amylase inhibitory assay, commonly used to evaluate the potential of substances to inhibit the enzyme alpha-amylase, was initially developed and described by Bernfeld in 1955. This assay has since been widely utilized in various studies, particularly in the context of diabetes research and the development of antidiabetic therapies, where the inhibition of alpha-amylase can slow down carbohydrate digestion and glucose absorption. The α-glucosidase inhibitory activity was evaluated using a modified version of the standard method (Widowati et al., 2011; Widowati et al., 2015; Soeng et al., 2015).

PHYTOCHEMICAL ANALYSIS

DPPH RADICAL SCAVENGING ACTIVITY; The DPPH method is a quick, straightforward, precise, and cost-effective assay used to assess the capacity of various compounds to function as free radical scavengers or hydrogen donors, making it valuable for evaluating the antioxidant activity of foods and beverages (Perez et al., 1990).

TOTAL PHENOLIC CONTENT; The Folin-Ciocalteu method is a widely used colorimetric assay to measure the total phenolic content in a sample. This method is based on a redox reaction between phenolic compounds and the Folin-Ciocalteu reagent, followed by an oxidation step in an alkaline environment. The resulting blue complex, which forms during the reaction, absorbs light at around 765 nm, with the intensity correlating to the phenolic content in the sample (Singleton et al., 1999)

TOTAL FLAVANOID CONTENT

Total flavonoid content of the methanolic extract of *Hibiscus cannabinus* leaves were determined according to a modified colorimetric method (Samuel et al., 2013). The determination of total flavonoid content using the aluminium chloride (AlCl3) colorimetric assay is based on the formation of a complex between flavonoids and aluminium chloride in the presence of a sodium acetate buffer at a pH of 5.5. This complex exhibits maximum absorption at approximately 415 nm.

TOTAL TANNIN CONTENT

The determination of total tannin content using the Folin-Ciocalteu reagent is a widely used method in plant extract analysis, by modification on the procedure from a paper published on Determination of Tannins and Their Correlations with Chemical and Protein Precipitation Methods. Journal of Agricultural and Food Chemistry (Makkar et al., 1993).

TOTAL ALKALOID CONTENT

The Total Alkaloid Content (TAC) test is a method used to quantify the alkaloid content in plant extracts. Alkaloids, which are nitrogen-containing compounds, are known for their pharmacological effects, making this test crucial in assessing the medicinal value of plants. The test typically involves the extraction of alkaloids using acidic or organic solvents, followed by precipitation with reagents like bromocresol green or Dragendorff's reagent (John et al., 2014).

RESULTS

1.1. PHYTOCHEMICAL ANALYSIS

TOTAL PHENOLIC CONTENT

Total phenolic content (TPC) of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus* leaves were estimated by Folin-Ciocalteau method using Gallic acid as standard. The TPC was calculated using based on the linear regression equation derived from Gallic acid.

Table 3: Total Phenol content (TPC) of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus*

SL.NO.	SAMPLE	FINAL CONC. Of TPC in mg GAE/g
1	HC. AQUEOUS	201.6±17.1
2	H.C ACETONE	159.2±13.6
3	H.C METHANOL	120.6±9.2
4	H.C 1:1 ACETONE: METHANOL	234.9±8.6

TOTAL FLAVONOID CONTENT

Total flavonoid content of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus* leaves were determined according to a modified colorimetric method, with Quercetin serving as the standard.

The TFC was calculated based on the linear regression equation derived from ascorbic acid.

Table 4: Total Flavonoid content (TFC) of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus*

SL.NO.	SAMPLES	FINAL CONC. Mg QE/g
1	HC. AQUEOUS	757.6±41.0
2	HC. METHANOL	690.3±45.1
3	HC. ACETONE	782.3±96.8
4	HC. 1:1 ACETONE AND METHANOL	885.6±24.6

TOTAL FLAVANOID CONTENT

Total flavonoid content of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus* leaves were determined according to a modified colorimetric method, with Quercetin serving as the standard. The TFC was calculated based on the linear regression equation derived from ascorbic acid

Table 5: Total Tannin content (TTC) of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts

SL.NO.	SAMPLES	FINAL CONC. of TTC in mg TAE/gm
1	HC. AQUEOUS	3906.3±37.7
2	HC. METHANOL	3962.1±3.8
3	HC. ACETONE	3810.4±21.6
4	HC. 1:1 ACETONE AND METHANOL	3981.3±17.5

DPPH RADICAL SCAVENGING ACTIVITY

The total antioxidant capacity (TAC) of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus* leaves was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method, with ascorbic acid serving as the standard. The TAC was calculated based on the linear regression equation derived from ascorbic acid.

Table 6: Radical scavenging activity of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus*

SL.NO.	SAMPLES	CONCENTRATION (µl/ml)	RADICAL SCAVENGING ACTIVITY (RSA) %
1	HC. AQUEOUS	200	97.93
		500	97.37
		1000	96.69
2	HC. METHANOL	200	95.51
		500	94.11
		1000	93.28
3	HC. ACETONE	200	91.91
		500	91.29
		1000	89.89
4	HC. 1:1 ACETONE AND METHANOL	200	40.86
		500	42.15
		1000	47.2

Table 7: IC50 values of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus*

SL.NO.	SAMPLES	IC50 (mg/ml)
1	HC. AQUEOUS	67.9
2	HC. METHANOL	38.5
3	HC. ACETONE	43
4	HC. 1:1 ACETONE AND METHANOL	25

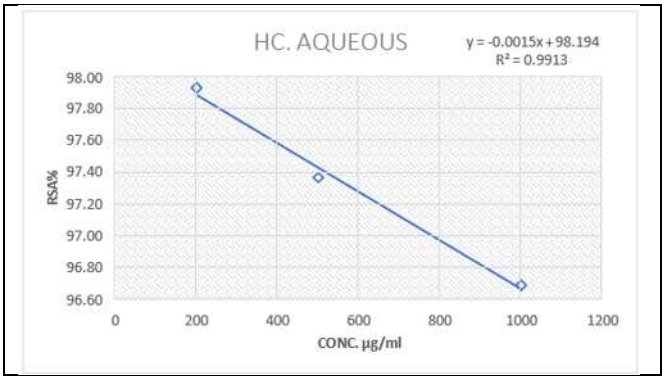


Fig 3: Graph showing Radical Scavenging Activity of *Hibiscus cannabinus* aqueous extract by DPPH Assay

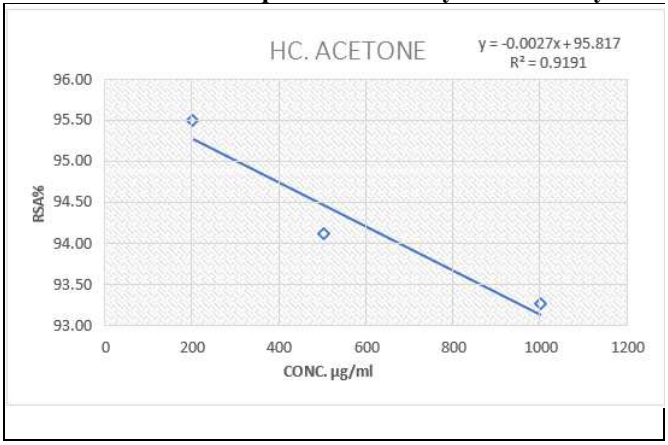


Fig 4: Graph showing Radical Scavenging Activity of *Hibiscus cannabinus* acetone extract by DPPH Assay

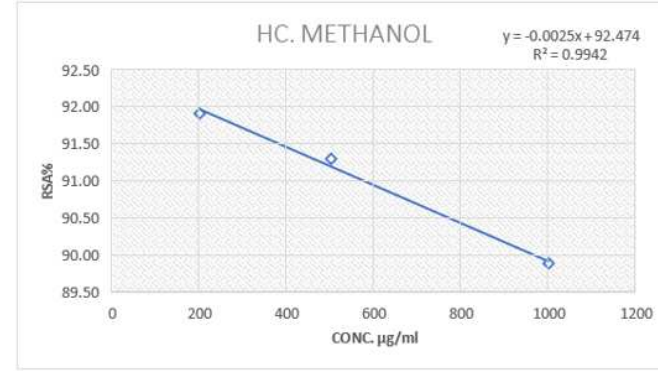


Fig 5: Graph showing Radical Scavenging Activity of *Hibiscus cannabinus* methanol extract by DPPH Assay

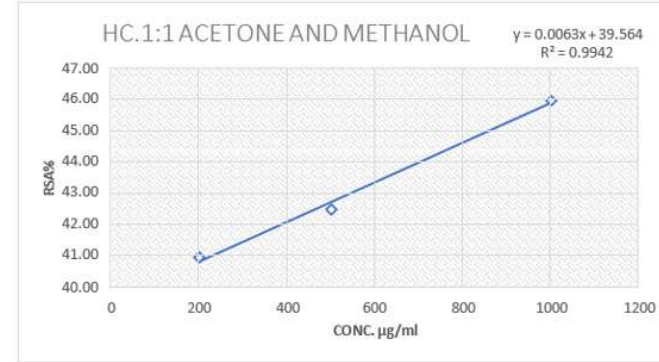


Fig 6: Graph showing Radical Scavenging Activity of *Hibiscus cannabinus* 1:1 acetone and methanol extract by DPPH Assay

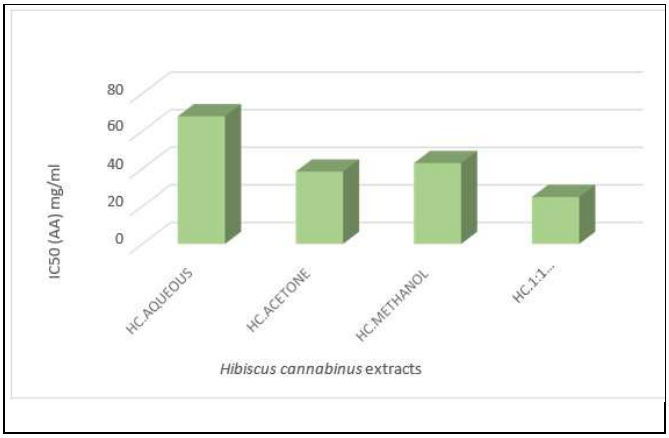


Fig 7: Comparation of IC50 values between all the extracts of Hibiscus cannabinus

TOTAL ALKALOID CONTENT

Total alkaloid content of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus* leaves were determined according to a modified colorimetric method, with Quercetin serving as the standard. The TAC was calculated based on the linear regression equation derived from ascorbic acid.

Table 8: Total Alkaloid content (TAC) of the aqueous, acetone, methanolic and 1:1 of the acetone and methanol extracts of *Hibiscus cannabinus*

SL.NO.	SAMPLE	FINAL CONC. mg QE/g
1	HC. AQUEOUS	2897.0±6.6
2	HC. ACETONE	2789.5±36.1
3	HC. METHANOL	2559.5±11.5
4	1:1 HC. ACETONE: METHANOL	4403.7±10.1

COMPARATION OF THE RESULTS ALL THE PHYTOCHEMICALS (TPC, TFC, TTC, TAC) FOUND IN *Hibiscus cannabinus*

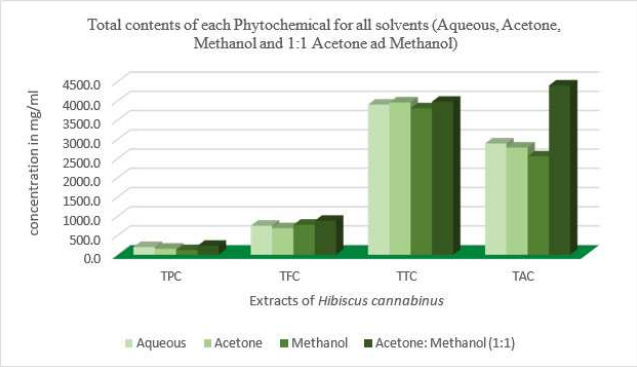


Fig 8: Comparison between all the phytochemical analysis for all solvents of *Hibiscus cannabinus*

Table 9: Comparation between all the phytochemical analysis for all solvents of

SAMPLE	TPC	TFC	TTC	TAC
HC. AQUEOUS	201.6±17.1	757.6±41.0	3906.3±37.7	2897.0±6.6
H.C ACETONE	159.2±13.6	690.3±45.1	3962.1±3.8	2789.5±36.1
H.C METHANOL	120.6±9.2	782.3±96.8	3810.4±21.6	2559.5±11.5
H.C 1:1 ACETONE: METHANOL	234.9±8.6	885.6±24.6	3981.3±17.5	4403.7±10.1

Hibiscus cannabinus

ANTIMICROBIAL ANALYSIS

The antimicrobial activity of aqueous, acetone, methanol and 1:1 of acetone and methanol extracts from *Hibiscus cannabinus* leaves was evaluated against bacterias such as *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* and a fungus that is *Candida albicans* using the agar-well diffusion method, with slight modifications

Table 10: Results and observation of antimicrobial activity against different solvent extracts of *Hibiscus cannabinus* leaves.

Sl. No.	Microorganism	Plant name	Plant part	Solvent	Conc. of samples	Amount of sample in each well (µl)	Zoi ± SD
1	***Escherichia coli***	***Hibiscus cannabinus***	Leaves	Aqueous	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				Acetone	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				Methanol	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				1:1 Acetone: Methanol	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
2	***Bacillus subtilis***	***Hibiscus cannabinus***	Leaves	Aqueous	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				Acetone	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				Methanol	ml	0	
					4mg/ml	100	--
3	***Staphylococcus aureus***	***Hibiscus cannabin***	Leaves		1mg/ml	100	--

		us		**Aqueous**	2mg/ml	100	--
					4mg/ml	100	--
				Acetone	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				Methanol	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				1:1 Acetone: Methanol	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
4	***Pseudomonas aeruginosa***	***Hibiscus cannabinus***	**Leaves**	**Aqueous**	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				Acetone	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				Methanol	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				1:1 Acetone: Methanol	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
5	***Candida albicans***	***Hibiscus cannabinus***	**Leaves**	**Aqueous**	1mg/ml	100	--
					2mg/ml	100	--
					4mg/ml	100	--
				Acetone	1mg/ml	100	--
					2mg/ml	100	13.6±0.6

				4mg/ml	100	15.3±1.5
			Methanol	1mg/ml	100	--
				2mg/ml	100	--
				4mg/ml	100	--
			1:1 Acetone: Methanol	1mg/ml	100	--
				2mg/ml	100	13.6±1.5
				4mg/ml	100	16.6±0.6

Table 11: Results and observation of negative controls

Microorganisms	**Zone of Inhibition [mm]**			
Sl. No.	**Aqueous**	**Acetone**	**Methanol**	**1:1 Acetone: Methanol**
1. *Staphylococcus aureus*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2. *Escherichia coli*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3. *Candida albicans*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4. *Pseudomonas aeruginosa*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5. *Bacillus subtilis*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 12: Results and observation of positive controls – Streptomycin and Ciprofloxacin

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- Each table representing the zone of inhibition has the average result of the triplicates of zone of inhibition for each concentration against each test microorganism as well as both positive and negative controls.
- "0.00 ± 0.00" indicates no zone of inhibition.

Microorganisms	**Zone of Inhibition [mm] ± Standard deviation**	
Sl. No.	**Streptomycin [100µg/ml]**	**Ciprofloxacin [4µg/ml]**
1. *Staphylococcus aureus*	32.3±0.58	-
2. *Escherichia coli*	31.3±1.58	-
3. *Candida albicans*	-	43.60 ± 2.60
4. Pseudomonas aeruginosa	31.3±0.58	-
5. Bacillus subtilis	33.3±1.0	-

Table 13: The zone of inhibition of *Escherichia coli* against different concentrations of *Hibiscus cannabinus* leave extract:

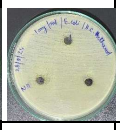
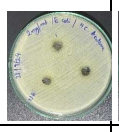
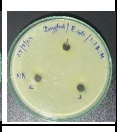
Sl. No.	**Microorganism**	**Concentrations**	**Aqueous extract**	**Acetone extract**	**Methanol extract**	**1:1 Acetone: Methanol**
1	***Escherichia coli*** **(Gram negative)**	**1 mg/ml**				
		2mg/ml				
		4mg/ml				

Table 14: The zone of inhibition of *Bacillus subtilis* against different concentrations of *Hibiscus cannabinus* leave extract:

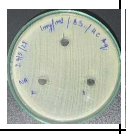
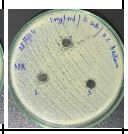
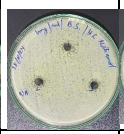
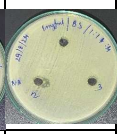
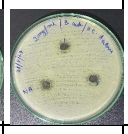
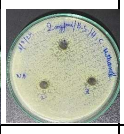
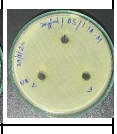
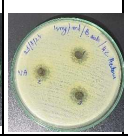
Sl. No.	**Microorganism**	**Concentrations**	**Aqueous extract**	**Acetone extract**	**Methanol extract**	**1:1 Acetone: Methanol**
1	***Bacillus subtilis*** **(Gram positive)**	**1mg/ml**				
		2mg/ml				
		4mg/ml				

Table 15: The zone of inhibition of *Staphylococcus aureus* against different concentrations of *Hibiscus cannabinus* leave extract:

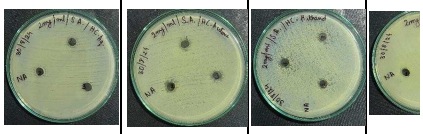
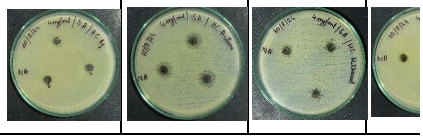
Sl. No.	Microorganism	Concentrations	Aqueous extract	Acetone extract	Methanol extract	1:1 Acetone Methan
1	*Staphylococcus aureus* (Gram positive)	1mg/ml				
		2mg/ml				
		4mg/ml				

Table 16: The zone of inhibition of *Pseudomonas aeruginosa* against different concentrations of *Hibiscus cannabinus* leave extract:

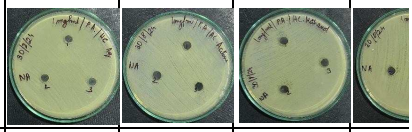
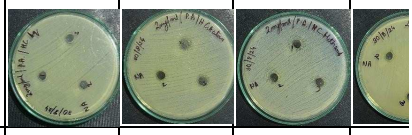
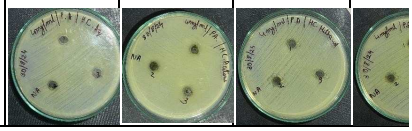
Sl. No.	Microorganism	Concentrations	Aqueous extract	Acetone extract	Methanol extract	1:1 Acet Meth extra
1	*Pseudomonas aeruginosa* (Gram negative)	1mg/ml				
		2mg/ml				
		4mg/ml				

Table 17: The zone of inhibition of *Candida albicans* against different concentrations of *Hibiscus cannabinus* leave extract:

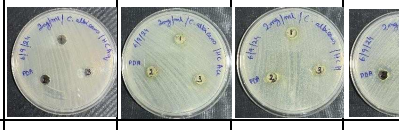
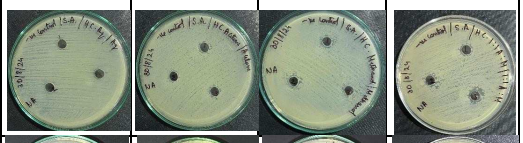
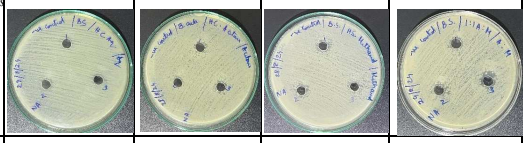
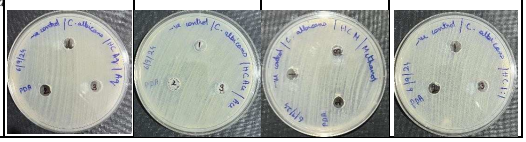
Sl. No.	Microorganism	Concentrations	Aqueous extract	Acetone extract	Methanol extract	1:1 Acet Meth extra
1	*Candida albicans*	1mg/ml				
		2mg/ml				
		4mg/ml				

Table 18: The zone of inhibition of different microorganisms against negative controls of different solvents:

Microorganism	Zone of Inhibition [mm] Aqueous	Acetone	Methanol	1:1 Acetone and methanol
1. *Staphylococcus aureus*				
2. *Escherichia coli*				
3. *Bacillus subtilis*				
4. *Pseudomonas aeruginosa*				
5. *Candida albicans*				

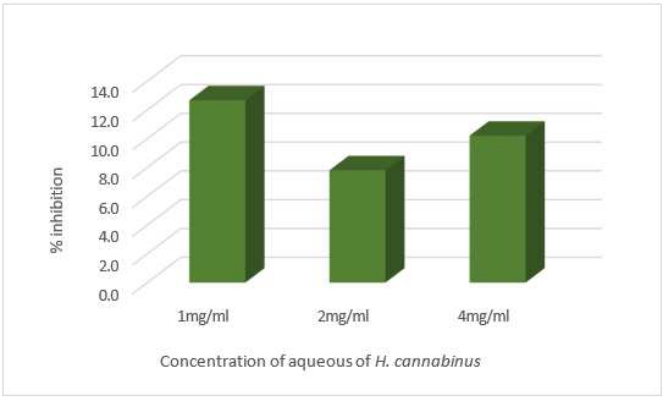
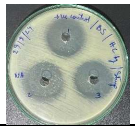


Table 19: Zone of inhibition of different microorganisms against of different antibiotics- Streptomycin and Ciprofloxacin

	Zone of Inhibition [mm]	
Sl. No.	**Streptomycin (100µg/ml)**	**Ciprofloxacin (4µg/ml)**
1. *Staphylococcus aureus*		-
2. *Escherichia coli*		-
3. *Bacillus subtilis*		-
4. *Pseudomonas aeruginosa*		-
5. *Candida albicans*	-	

ALPHA AMYLASE INHIBITORY ACTIVITY

The potential of *Hibiscus cannabinus* leaf extracts to inhibit α-amylase activity was assessed using different solvent extracts at concentrations of 1 mg/ml, 2 mg/ml, and 4 mg/ml. Based on the diameter of the hydrolysis zones around the wells, the percentage inhibition of α-amylase was calculated. The highest inhibition observed was at the 1 mg/ml concentration, showing 12.7% inhibition. The results suggest a moderate ability of the extracts to inhibit the enzyme, with variation depending on the concentration and solvent used.

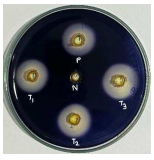
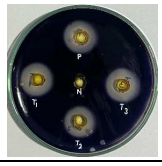
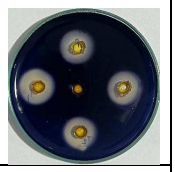
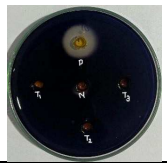
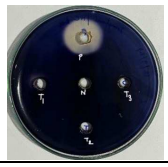
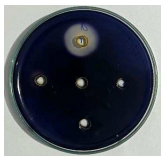
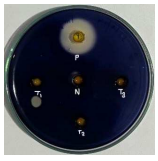
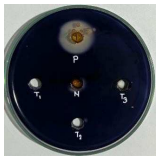
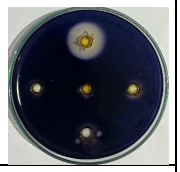
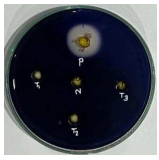
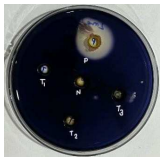
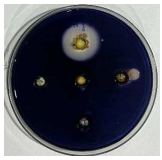
Solvent extract of acetone, methanol and 1:1 of acetone methanol might show amylase inhibition but due to solvent interaction with enzyme it cannot be confirmed with this test.

Table 20: Percentage inhibition of α-amylase

Hibiscus cannabinus extracts	Concentrations	Percentage inhibition (%)
Aqueous	1mg/ml	12.7
	2mg/ml	7.8
	4mg/ml	10.2

Fig 9: Percentage inhibition of α-amylase

Table 21: *Hibiscus cannabinus* extracts showing alpha amylase activity against different solvents

Sl. No.	*Hibiscus cannabinus* extracts	Zone of Inhibitions (positive control, negative control, and samples-T1, T2, T3)		
		1mg/ml	2mg/ml	4mg/ml
1	Aqueous			
2	Acetone			
3	Methanol			
4	1:1 acetone and methanol			

DISCUSSION

In our present study, we investigated the phytochemical composition and biological activities of Hibiscus cannabinus L. leaf extracts prepared using four different solvent systems: water, acetone, methanol, and a 1:1 mixture of acetone and methanol. We selected these solvents to examine how the extraction medium influenced the recovery of leaf constituents and the subsequent antioxidant and enzyme-inhibitory activities of the extracts. The dried extract weights recorded in our study were 3.173 g for the aqueous extract, 1.775 g for the acetone extract, 2.18 g for the methanol extract, and 2.8 g for the acetone–methanol extract. Based on these recorded weights, the aqueous extract yielded the greatest mass of dried material,

followed by the acetone–methanol, methanol, and acetone extracts.

Our observations indicate that the choice of solvent influenced the amount of material recovered from the powdered leaves. However, we recognise that extraction yield alone does not establish the concentration of individual phytochemicals or the biological effectiveness of an extract. The mass of dried material recovered represents the overall extractable fraction obtained under the conditions employed, whereas the phenolic, flavonoid, tannin, antioxidant, and enzyme-inhibitory measurements provide separate information about particular chemical or biological properties. Therefore, we considered the extraction yield alongside the other parameters rather than using it as the sole indicator of extract quality.

Our findings can be compared with those of Yusri et al. (2012), who investigated the phenolic content and antioxidant activity of Hibiscus cannabinus seed extracts following sequential solvent extraction. They reported extraction yields ranging from 4.63% to 16.60%, with hexane producing the highest yield. Nevertheless, their aqueous seed extract showed the highest activity in several antioxidant assays, including DPPH radical scavenging, despite not producing the greatest extraction yield. This observation is relevant to our study because it demonstrates that the solvent yielding the largest amount of extract need not necessarily produce the extract with the strongest measured biological activity. Although Yusri et al. examined seeds rather than leaves and used a different extraction procedure, their results support our approach of evaluating extraction yield and biological activity as separate parameters.

Similarly, Ryu et al. (2017) demonstrated that phytochemical composition in kenaf varied according to plant tissue and extraction solvent. Their investigation included leaves, flowers, seeds, and stem bark, and they reported differences in phenolic and flavonoid contents among these tissues and their corresponding extracts. Their observations indicate that the chemical characteristics of H. cannabinus cannot be considered independent of the extraction conditions and plant material. In our study, the use of different solvents likewise produced extracts with different measured phytochemical contents and antioxidant responses.

The results reported by Sulaiman et al. (2024) also provide a relevant comparison. They found that water was effective for extracting phenolic and flavonoid constituents from kenaf, with leaves showing high phenolic content. In contrast, the acetone–methanol mixture produced the highest recorded total phenolic content, total flavonoid content, and tannin content in our study. We interpret this difference in relation to the distinct extraction procedures and experimental conditions used in the respective investigations. The findings do not necessarily indicate that one extraction solvent is universally superior, because the recovery of measured constituents may depend on the plant material, extraction procedure, solvent system, and analytical method.

In our study, we determined the total phenolic content (TPC) of the four H. cannabinus leaf extracts using the Folin–Ciocalteu method. Among the extracts examined, the acetone–methanol mixture showed the highest recorded TPC of 234.9 ± 8.6, followed by the aqueous extract at 201.6 ± 17.1, the acetone extract at 159.2 ± 13.6, and the methanol extract at 120.6 ± 9.2. Based on these measurements, the acetone–methanol mixture produced the highest TPC value under our experimental conditions, whereas the methanol extract produced the lowest.

We interpret this finding as evidence that the mixed-solvent system recovered a greater amount of material contributing to the measured phenolic response than the other tested solvents. Nevertheless, we do not interpret the result as proof that the mixed-solvent extract contained the greatest quantity of every individual phenolic compound. The Folin–Ciocalteu assay provides an overall measurement of the response attributed to phenolic content under the conditions used; it does not identify the individual molecules responsible for that response. Accordingly, the chemical identity and relative abundance of specific phenolic compounds in our extracts cannot be established from TPC measurements alone.

Our findings can be compared with those of Birhanie et al. (2021), who evaluated the phenolic content of leaves from 33 H. cannabinus genotypes. They reported TPC values ranging from 6.03 to 21.15 mg gallic acid equivalents (GAE)/g dry weight. Their study demonstrated variation in phenolic content among kenaf genotypes, indicating that the measured phenolic composition may differ even within the same plant species. In our investigation, we focused on differences among solvent extracts prepared from the collected leaf material.

Ryu et al. (2017) also reported variation in phenolic content among kenaf tissues and extraction solvents. In their study, the aqueous leaf extract showed a reported TPC of 555.9 mg/100 g, while the methanolic leaf extract showed 178.1 mg/100 g. They also found high phenolic values in some water extracts from flowers and seeds. These observations demonstrate that both plant tissue and extraction medium can influence the measured phenolic content. In our study, however, the acetone–methanol mixture produced the highest recorded TPC, followed by the aqueous extract. This difference suggests that the relative performance of individual solvents may vary with the extraction procedure and experimental conditions.

Duan et al. (2024) further investigated phenolic content in kenaf shoots at different growth stages. They reported leaf TPC values ranging from 11.27 to 16.07 mg GAE/g dry weight, compared with 2.09 to 8.48 mg GAE/g dry weight in stems. Their findings indicated that phenolic content differed between leaves and stems and changed during plant development. This is relevant to our work because it highlights the importance of specifying the plant tissue and sampling conditions when interpreting phytochemical measurements.

Sulaiman et al. (2024) also reported high phenolic content in kenaf leaves and identified water as an effective solvent for recovering phenolic constituents in their experimental system. The relatively high TPC recorded for our aqueous extract is consistent with the general observation that water can recover measurable phenolic constituents from kenaf leaves. At the same time, our acetone–methanol extract produced the highest TPC in the present experiment. We consider this difference to reflect the solvent-specific results obtained under the respective experimental procedures rather than evidence that one solvent must always yield the highest phenolic content.

In our study, we evaluated the total flavonoid content (TFC) of our H. cannabinus leaf extracts. The acetone–methanol extract showed the highest recorded value, at 885.6 ± 24.6, followed by the acetone extract at 782.3 ± 96.8, the aqueous extract at 757.6 ± 41.0, and the methanol extract at 690.3 ± 45.1. These findings indicate that the mixed-solvent extract produced the highest measured flavonoid response under our experimental conditions. The measured TFC does not establish the identity of individual flavonoids. Birhanie et al. (2021), Ryu et al. (2017), Duan et al. (2024), and Sulaiman et al. (2024) reported variation in flavonoid content with genotype, tissue, growth stage, and extraction conditions.

The acetone–methanol extract showed the highest recorded tannin value (3981.3 ± 17.5), followed by methanol (3962.1 ± 3.8), aqueous extract (3906.3 ± 37.7), and acetone extract (3810.4 ± 21.6). The mixed-solvent and methanol values were relatively close, so we should avoid describing their difference as substantial without appropriate statistical testing. Okereke et al. (2015) and Hamrita et al. (2022) reported tannins among the phytochemical constituents of H. sabdariffa calyx extracts.

The aqueous extract showed DPPH inhibition values of 97.93%, 97.37%, and 96.69% at 200, 500, and 1000 µL, respectively. The methanol extract showed 95.51%, 94.11%, and 93.28%, while the acetone extract showed 91.91%, 91.29%, and 89.89%. The acetone–methanol extract showed lower reported values of 40.86%, 42.15%, and 47.20%. Thus, the aqueous extract showed the highest reported DPPH percentages, despite the acetone–methanol extract having the highest recorded TPC, TFC, and tannin values. Yusri et al. (2012) also reported the aqueous seed extract as strongest in several antioxidant assays. Ryu et al. (2017), Birhanie et al. (2021), and Duan et al. (2024) showed that antioxidant responses can vary with tissue, solvent, genotype, and growth stage.

Our study examined the antibacterial activity of H. cannabinus leaf extracts against S. aureus, E. coli, P. aeruginosa, and B. subtilis. Birhanie et al. (2021) and Sulaiman et al. (2024) reported antibacterial effects associated with kenaf leaf material. Kumari and Gowda (2021) reported differences among Hibiscus species, solvents, and test organisms. Bindhu et al. (2014) and Joe Princy et al. (2023) studied nanoparticles made using H. cannabinus plant material. Studies on H. sabdariffa calyces and flowers provide related background.

Wali et al. (2024) reported inhibition zones for several fungi using an acetone extract of H. sabdariffa stems, while Venkatesan et al. (2024) examined H. sabdariffa methanolic extract against selected microorganisms including C. albicans. Hamrita et al. (2022) also examined antimicrobial properties of H. sabdariffa calyx extracts.

We evaluated α-amylase inhibition at 1, 2, and 4 mg/mL. The aqueous extract showed 12.7%, 7.8%, and 10.2% inhibition, respectively. The response was not consistently concentration-dependent, and the highest recorded inhibition was 12.7% at 1 mg/mL. Vo et al. (2025) reported α-amylase IC50 values of 1.39 ± 0.09 mg/mL for aqueous and 0.95 ± 0.06 mg/mL for 70% ethanol extracts of H. rosa-sinensis flowers. They also reported α-glucosidase inhibition. Hansawasdi et al. (2000) identified hibiscus acid and its 6-methyl ester as active principles in roselle tea.

Studies on H. sabdariffa by Okereke et al. (2015), Hamrita et al. (2022), and Hagr and Adam (2020) reported phytochemical constituents in calyces or seeds. Obidoa et al. (2011), examined aqueous H. sabdariffa flower extract. Ryu et al. (2017) and Duan et al. (2024) used compound-specific analyses to examine phenolic compounds in kenaf. Our total-content assays are preliminary measures and do not identify every compound or its specific biological role.

CONCLUSION

The present study investigated the phytochemical composition and selected biological activities of aqueous, acetone, methanol, and acetone–methanol extracts of Hibiscus cannabinus L. leaves. The findings revealed variations in extraction yield, total phenolic content, total flavonoid content, and total tannin content among the different solvent extracts. Among the tested extracts, the 1:1 acetone–methanol extract exhibited the highest reported values for total phenolic, flavonoid, and tannin contents, demonstrating the influence of solvent

selection on the extraction of phytochemical constituents from the leaves.

The study also investigated the antibacterial, antifungal, and α-amylase inhibitory properties of the extracts. The aqueous extract exhibited α-amylase inhibitory activity at the tested concentrations. The overall findings demonstrate that H. cannabinus leaves contain measurable phytochemical constituents and provide a basis for understanding the biological properties of extracts prepared using different solvents.

In conclusion, this study contributes to the phytochemical investigation of Hibiscus cannabinus L. and highlights the importance of solvent selection when evaluating its leaf extracts. The findings provide a foundation for further exploration of the plant's potential applications in phytochemical and biological research.

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