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Evaluation of *In-vitro* Antibacterial Potentialities Against Some Pathogenic Bacteria and Bioprospecting of *Cleistanthus Collinus* (Roxb.) Benth.ex Hook.f.

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ABSTRACT *Cleistanthus collinus* (Roxb.) Benth.ex Hook. f., a member of the Euphorbiaceae family, exhibits significant therapeutic potential in the field of phytochemical research, notwithstanding its prevalent use in suicidal contexts. The primary objective of this research is to systematically analyze the phytochemical composition of various solvent extracts including petroleum ether, chloroform, ethyl acetate, methanol, and water obtained from diverse plant parts, namely leaves, bark, and fruits of *C. collinus*. Subsequently, the obtained extracts underwent evaluation for their antibacterial efficacy against a selected panel of Gram-positive bacteria, including *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, as well as Gram-negative bacteria, comprising *Escherichia coli*, *Salmonella paratyphi*, *Shigella dysenteriae*, and *Vibrio cholera*. Notably, the methanol and ethyl acetate extracts demonstrate pronounced antibacterial activity, surpassing that observed with chloroform extracts. The current study discovered that *C. collinus* extracts contained a range of secondary metabolites and it has the potential to be utilized in the production of pharmaceutical drugs, notably antibacterial ones.

INTRODUCTION

The historical significance of therapeutic plants, rooted in centuries of traditional use, underscores their pivotal role as the precursors to modern medicine. Over time, plant-derived chemicals have consistently emerged as crucial sources of bioactive molecules, serving as foundational components in the development of pharmaceuticals. Plants have been traditionally utilized in medicines since time immemorial and are being used now. The usage of these plants has been increasingly polished through centuries, and it is now recognized as traditional medicine in many instances (Esther et al. 2020). Natural remedies have been widely utilized to improve good health from the time since antiquity and the efficacy of current clinical trials is heavily reliant on pharmaceuticals derived from nature's bounty. To manage and treat infectious pathogens, an enormous variety of antimicrobial agents were developed in the past from chemical-based and organic sources (Taye et al. 2021).

Infections caused by microbial agents and the rise of drug-resistant microorganisms have emerged as major global issues. Microbial-related illnesses kill millions of people each year due to a scarcity of potent medications in markets and the adverse consequences of antibiotics that are synthetic. Therapeutic plants are abundant in biologically active substances, which include a wide range of phytochemicals and modes of action. Numerous phytochemicals have demonstrated effective antimicrobial activity (Saravanan et al. 2021). One approach to addressing this issue is to look for novel possible antimicrobial agents in plants. Plants have been utilized as remedies for humans since earlier times. Over 80 percent of individuals in the globe, particularly those who reside in rural regions, utilize medications derived from herbal sources (Mukherjee 2002; Bodeker et al. 2005; Bandaranayake 2006). Numerous investigations have highlighted plants as the origin for the growth of the vast majority of presently used traditional drugs, as well as the possibilities for the upcoming discovery of innovative agents against a variety of illnesses. The bulk of medications now in circulation are derivative products of secondary plant compounds discovered from crude extracts (Tewodros and Yared 2020).

The genus *Cleistanthus* encompasses more than 140 species, with *Cleistanthus collinus* being particularly noteworthy due to its toxic nature,

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often employed in homicidal or suicidal incidents (Jain et al. 2010). Thriving in the tropical landscapes of southern and central India, the plant under investigation, identified as vulnerable by the International Union for Conservation of Nature (IUCN) (Misbah and Alok 2022), holds ecological significance warranting a comprehensive exploration of its phytochemical composition and potential therapeutic applications. *C. collinus* is characterized by a rich reservoir of bioactive compounds. The roots, leaves, and fruits exhibit astringent properties, with alcoholic extracts traditionally utilized for the treatment of gastrointestinal disorders. These extracts, upon investigation, have demonstrated noteworthy anticancer activity. Moreover, they find application as cleansing agents for septic wounds and exhibit efficacy against fungal diseases. The leaves, on the other hand, possess antifungal properties (Remya et al. 2018). *C. collinus* is known to contain lignans, such as cleistanthin A and B, which exhibit cytotoxic, antitumor, and larvicidal properties (Ramaraj et al. 2015). In the pursuit of unveiling the therapeutic potential of *C. collinus*, the present study embarks on a comprehensive exploration, encompassing a preliminary phytochemical screening and the assessment of antibacterial activity in diverse extracts derived from its leaves, bark, and fruits. This research contributes to a deeper understanding of the plant's chemical composition and potential pharmacological applications.

Objective

The current research is mainly concerned with two major objectives:

1. To qualitatively analyze and screen possible phytocompounds in various solvent extracts obtained from leaves, bark and fruits of *C. collinus*.
2. Evaluation of these extracts on antimicrobial activities against a variety of Gram-positive and Gram-negative pathogenic bacteria.

MATERIALS AND METHODS

Sample Collection

Cleistanthus collinus leaves, bark, and fruits were manually collected in January 2022 from the geographical coordinates 17^{0}22' 09.16" N and 81^{0}47' 48.42" E near Gopavaram village, Andhra Pradesh, India. The authentication process was carried out by a taxonomist affiliated with the Department of Botany at Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The specimens obtained were assigned the accession number SVUH (Sri Venkateswara University Herbarium): 1226, ensuring their taxonomic validation.

Extraction of Plant Material

Plant materials obtained were desiccated in shaded conditions at 32°C and subsequently pulverized into powder. To ensure uniform particle size, the powder underwent sieving using a 0.5 mm sieve and was stored in sterile containers for later use. Utilizing a spectrum of solvents, namely petroleum ether, chloroform, ethyl acetate, methanol, and water, the extraction process targeted the leaves, bark, and fruits of *C. collinus* to capture a diverse array of compounds, forming the basis of the investigation's exploration into the phytochemical composition of this plant. The phytochemicals were extracted from the plant material using the specified solvents in a soxhlet extractor (Harborne 1984). Upon collection, the extracts were concentrated into a thick consistency using a cold distillation procedure.

Preliminary Phytochemical Screening

The standard protocols (Vishnu et al. 2019) were followed to conduct a preliminary phytochemical analysis using the crude extracts.

The extraction values were calculated using the formula shown below:

Yield (%) = W_1/W_1 x 100

Wherein, W_1 is the extract's weight after the solvent has evaporated and W_2 is the dry weight of the plant sample.

Collection of Test Pathogens

A total of eight test bacteria, of which four were gram-negative *Shigella dysenteriae* (ATCC 13313), *Salmonella enterica ser. Paratyphi* (MTCC 735), *Vibrio cholera* (MTCC 3906) and *Escherichia coli* (MTCC 443), whereas four gram-positive *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 2940), *Micrococcus luteus* (MTCC 2452) and *Staphylococcus epidermidis* (MTCC 3615), were utilized in the present study. All these bacte-

rial cultures were acquired from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology (IMTECH), Chandigarh, India and one from the American Type Culture Collection (ATCC), Bangalore, India.

Analysis of Antibacterial Activity of Crude Plant Extracts

To evaluate the antibacterial potential of the plant extracts, the agar well diffusion technique was employed on Nutrient Broth (NB) agar medium. After the preparation and solidification of the nutrient agar in Petri plates, a sterile L-shaped bent rod facilitated the uniform distribution of a 100 µL bacterial culture. Wells, created with a 5 mm diameter sterile cork borer, were subsequently filled with various solvent extracts (10 mg, 5 mg, and 2.5 mg in 10% aqueous Dimethyl sulfoxide (DMSO)), along with positive and negative controls (20 µL each). Incubation at 37 °C for 24 hours preceded the measurement of zones of inhibition (mm) produced by the different extracts. Positive controls, Streptomycin, and Penicillin, were set at a concentration of 100 µg/mL for gram-negative and gram-positive bacteria, respectively, while DMSO (10%) served as the negative control. Following three repetitions, the outcomes of each test were averaged, while maintaining a strictly aseptic environment throughout the microbiological experiment with meticulous care.

RESULTS AND DISCUSSION

Soluble Compound Percentages of Different Solvent Extracts

Table 1 shows the proportions of soluble components in various solvent extracts of *C. collinus* leaf, bark, and fruit. On account of leaf, water extract has the most elevated extent of dissolvable chemicals (18.92%), trailed by methanol (15.92%) though petroleum ether delivered the least yield (10.3%), and followed by ethyl acetate (11.5%). Similarly, water has the most noteworthy level of dissolvable compounds (19.01%) in bark separates in the above solvents, trailed by methanol (16.29%) and petroleum ether yielded the least (7.22%), trailed by ethyl acetate (10.81%). In the case of fruit extracts, the water showed the highest percentage of soluble compounds (16.90%), followed by methanol (11.84%) whereas the least yield was accounted for in petroleum ether (8.11%), followed by ethyl acetate (8.92%).

Preliminary Qualitative Phytochemical Evaluation

The results of the qualitative screening performed on the various *C. collinus* extracts revealed

Table 1: Percentages of the soluble compounds in leaf, bark and fruit extracts

Plant part used for Extraction Percentage of the extract		*The solvent used for extraction*	*Weight of the powered material*	*The volume of the solvent*	*Weight of the soluble extract*
Leaf	Methanol	30 gm	250 ml	4.776	15.92
	Ethyl acetate	30 gm	250 ml	3.45	11.5
	Chloroform	30 gm	250 ml	4.44	14.8
	Aqueous	30 gm	250 ml	5.679	18.92
	Petroleum ether	30 gm	250 ml	3.09	10.3
Bark	Methanol	30 gm	250 ml	4.875	16.25
	Ethyl acetate	30 gm	250 ml	3.243	10.81
	Chloroform	30 gm	250 ml	4.56	15.2
	Aqueous	30 gm	250 ml	5.73	19.1
	Petroleum ether	30 gm	250 ml	2.16	7.22
Fruit	Methanol	30 gm	250 ml	5.07	16.9
	Ethyl acetate	30 gm	250 ml	2.67	8.92
	Chloroform	30 gm	250 ml	3.24	10.8
	Aqueous	30 gm	250 ml	5.07	16.9
	Petroleum ether	30 gm	250 ml	2.43	8.11

Source: Authors

that they included phytosterols, glycosides, terpenoids, flavonoids, alkaloids, saponins, phenols carbohydrates, tannins, oils and amino acids. These results are shown in Table 2. In both methanol and ethyl acetate extracts, a wide range of secondary metabolites was present. Chloroform extract showed a medium assortment of these secondary metabolites. Contrasted with the remaining solvent extracts, petroleum ether and water extracts have the lowest number of secondary metabolites. However, phenols were detected in every one of the five leaf extracts. Likewise, saponins were identified in each of the five extracts in bark, but in the case of fruits 3 phyto-compounds in particular saponins, alkaloids and phenols are present in all five extracts. Methanol and ethyl acetate was found to be the most effective at extracting secondary metabolites from leaf and fruit concentrates, followed by water and petroleum ether, and chloroform was found to be the least effective whereas, in bark extracts, ethyl acetate extract dissolved the highest number of active principles, followed by methanol, chloroform, lastly petroleum ether and water.

In previous research, the ethanolic leaf extract of *C. collinus* was found to contain 8 different constituents, including alkaloids, cardiac glycosides, flavonoids, polyphenols, quinones, saponins, tannins and terpenoids with terpenoids and polyphenols being abundant while tannins were lacking (Arulmanickam and Chitra 2020). According to Suman and Elangomathavan (2013), alkaloids, flavonoids, glycosides, phlobatannins, saponins, steroids, tannins, and terpenoids have been identified in leaf extracts of *C. collinus*. Similarly, a study by Nayak et al. (2025) conducted a qualitative phytochemical screening of *C. collinus* fruits and bark, revealing the presence of tannins, saponins, flavonoids, phenolic compounds, reducing sugars, steroids, and alkaloids. These phytoconstituents are linked to medicinal properties, including antimicrobial, immunomodulatory, anticarcinogenic, antioxidant, and antihyperglycemic activities. The findings of our investigation align with prior reports, underscoring the efficiency of methanol as the most effective solvent for extracting secondary metabolites from *C. collinus*. This corroborates existing literature where methanol, followed by ethanol and ethyl acetate, exhibited superior extraction capabilities. Our study extends the understanding of the phytochemical profile of *C. collinus*, particularly in the aqueous leaf extract, obtained through sequential extraction with n-butanol, ethyl acetate, dichloromethane, and hexane. The comprehensive analysis revealed the presence of diverse phytochemical constituents, including tannins, terpenoids, flavonoids, saponins, glycosides, steroids, phlobatannins, and alkaloids, aligning with the findings reported by Ramaraj et al. (2015).

Additionally, this investigation resonates with independent studies conducted by Yugal et al. (2018), further validating the richness of phytoconstituents in *C. collinus*. The identification of significant compounds such as tannins, phenolic compounds, flavonoids, saponins, glycosides, and

Table 2: Preliminary qualitative phytochemical assay of various extracts of *C. collinus*

Test	*Leaf*					*Bark*					*Fruit*				
	Aq	*Me*	*Ea*	*Ch*	*Pe*	*Aq*	*Me*	*Ea*	*Ch*	*Pe*	*Aq*	*Me*	*Ea*	*Ch*	*Pe*
Carbohydrates	++	-	+	-	+	+	-	+	-	+	+	-	++	-	+
Amino acids	+	+	+	-	-	-	+	+	-	-	+	+	+	-	-
Oils	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+
Alkaloids	+	++	++	-	+	+	+	++	-	+	+	++	+	-	+
Flavonoids	++	-	+	-	+	-	-	+	+	+	+	+	+	+	+
Terpenoids	-	++	+	+	+	-	++	-	+	-	-	++	-	+	+
Phenols	+	++	+	+	+	-	++	+	+	+	+	++	+	+	+
Tannins	+	++	+	-	-	+	++	+	+	-	+	+	+	+	-
Phytosterols	-	++	+	+	-	+	++	+	-	-	+	++	-	-	+
Glycosides	+	++	-	+	-	+	++	+	+	-	-	-	+	+	-
Saponins	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+

Aq; Aqueous extract, Me; Methanol Extract, Ea; Ethyl acetate extract, Ch; Chloroform extract and Pe; Petroleum ether extract. (-): negative (absent), (+): Positive (slightly present), (++): Positive (Abundantly present)
*Source:*The authors give data from their analysis in the form of a table

triterpenoids in aqueous leaf extracts underscores the robust and consistent nature of the phytochemical composition across various investigations. Consistent outcomes were observed in the preliminary phytochemical analysis of leaf and bark extracts conducted by Jain et al. (2010), employing petroleum ether, benzene, chloroform, water, methanol, and ethyl acetate as solvents.

The analysis revealed the presence of alkaloids, coumarins, flavonoids, terpenoids, steroids, tannins, phenols, volatile oils, quinones, and cardiac glycosides, with the latter being present in all extracts. Notably, cardiac glycosides have been proposed as potential agents for cancer therapy. In contrast to previous studies, the latest investigation encompasses the phytochemical screening of *C. collinus* leaf, bark, and fruit extracts using five solvents: methanol, ethyl acetate, chloroform, petroleum ether, and water. The convergence of our results with existing literature strengthens the reliability and reproducibility of the reported phytochemical constituents in *C. collinus*, offering a comprehensive perspective for further research and development in the realm of natural products and medicinal plant utilization.

Antibacterial Activity

This study's investigation examined the antibacterial potential of crude extracts derived from different components of the *C. collinus* plant-leaves, bark, and fruit-with varying concentrations of 10 mg, 5 mg, and 2.5 mg, respectively. The antibacterial bioassay provided valuable insights into the inhibitory effects of these extracts against a spectrum of pathogens. The characterization of results relied on the measurement of the zone of inhibition, a crucial parameter indicating the extent of antibacterial activity. Chloroform extract had the lowest antibacterial activity, although methanol and ethyl acetate solvents displayed remarkable antibacterial activity.

Methanol extracts of leaf, bark and fruit showed significant antibacterial activity against every single tried bacterium. Particularly leaf methanol extracts showed the highest activity when compared to fruit and bark extracts. Despite being more potent than streptomycin (28 mm), methanol crude extract of leaf demonstrated high activity against *S. aureus*(35 mm), followed by *S. dysenteriae* (20.6 mm), *M. luteus* (19 mm), *V. cholera* (18.3 mm), and *B. subtilis* (17 mm) and moderate activity against the other four pathogens. Leaf extracts of ethyl acetate also showed notable effectiveness against *S. aureus* (29 mm) and moderate action against other bacterial pathogens (Tables 3 and 4, Figs. 1 and 2). In comparison to methanol and ethyl acetate extracts, chloroform extracts of leaves, bark and fruits had the least antibacterial effectiveness. Of the eight examined pathogens, *S. aureus* is the most susceptible to *C. collinus,* despite *S. epidermidis* being more resilient.

Furthermore, the antibacterial activities of plant extracts (10 mg) were compared to the inhibitory capability of the positive controls Streptomycin (gram-negative bacteria) and Penicillin (gram-positive bacteria) (Table 5). The leaf methanol extract of *C. collinus* showed the greatest percentage of inhibition compared to positive control (125%) against *S. aureus*, followed by fruit methanol (123.57%) and leaf ethyl acetate (103%). With the exception of the chloroform extract from the bark, the remaining eight plant extracts exhibited an inhibitory effect exceeding 50 percent against *Staphylococcus aureus* in comparison to the standard. An equal percentage of inhibition capability (103%) is seen against *S. dysenteriae* in the leaf methanol extract and against *S. aureus* in the leaf ethyl acetate extract. In each case, methanol extracts showed the highest rate of inhibition, but in the case of *S. epidermidis*, the percentage of inhibition by leaf ethyl acetate extract (51.85%) has more inhibition capacity than the leaf methanol extract (48.14%) of *C. collinus* plant.

The leaves, bark, and fruits of *C. collinus* showed higher antibacterial activity against *S. aureus*, *M. luteus*, *E. coli*, *V. cholera*, *S. dysenteriae*, and *B. subtilis* when extracted in methanol and ethyl acetate. The investigated pathogens could not be effectively controlled by *C. collinus* extracts in chloroform, although ethyl acetate and methanol extracts of the plant had stronger antimicrobial efficacy. Research indicates that numerous plant secondary metabolites play a crucial role in defending against microbial infections, pest attacks, and herbivory. These bioactive compounds, including catecholic coumarins, benzoxazinoids, terpenes, jasmonate, and indole glucosinolates, exhibit significant antimicrobial properties, enhancing plant resistance against bacterial and fungal pathogens (Wang et al. 2020; Maggini et al. 2018; Rajniak et al. 2018). The inhibiting effects of the

Table 3: Antibacterial activity (Gram-negative bacteria) of different extracts (ME, EA & CH) at the dosages of 10mg, 5mg, and 2.5mg

Plant part	*Extract*	*S. paratyphi*			*S. dysenteriae*			*E. coli*			*V. cholera*		
		10 mg	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*
Leaf	ME	17.6 ±0.5	13 ±1	11.3 ±0.5	20.6±0.57	17±1	14.3±0.57	15.6±0.5	13±1	12±0	18.3±0.57	17±0	13±1
	EA	10.3 ±0.5	9 ± 1	8.6 ±0.5	13±1	11.3±0.5	8.3±0.57	11±1	9±1	6.3±0.5	17.3±0.5	18±1	16.3±0.5
	CH	11.3 ±0.5	8.6 ±0.5	7 ±1	8.3±0.57	8±1	7.3±0.57	6.6±0.5	-	-	10.3±0.5	7.6±0.5	7.3±0.5
Bark	ME	13.6 ±0.5	13 ±1	8 ±1	13±1	10.6±0.5	8.3±0.57	21.6±0.5	17.3±0.5	11.3±0.5	16.6±0.5	13 ±1	11 ±1
	EA	8.6 ±0.5	7.6 ±0.5	6.6 ±0.5	9.3±0.57	8.6±0.57	7.6±0.57	14.6±0.5	12.6±0.5	9.3±0.5	13±1	12.3±0.5	10.3±0.5
	CH	7.6 ±0.5	.	.	.	.	.	6.6±0.5	.	.	7.3±0.5	.	.
Fruit	ME	14 ±1	14 ±0	10.6 ±0.5	17±1	16.3±0.5	12±1	23.6±0.5	18.3±0.5	15.6±0.5	16±0	12.3±0.5	7.6±0.5
	EA	8 ±1	7 ±1	7 ±1	8.3±0.57	7±0	18.3±0.5	13±1	11±1	9.3±0.5	12.3±1	11±1	8.3±0.5
	CH	10 ±1	9.6 ±0.5	8 ±1	8±1	7.6±0.57	7±0	6.6±0.5	.	.	8±0	7.3±0.5	.

Values represent mean plus standard deviations; ME; methanol extract, EA; ethyl acetate, CH; chloroform, and "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive
Source: The authors give data from their analysis in the form of a table

Table 4: Antibacterial activity (Gram-positive bacteria) of different extracts (Me, EA and Ch) at the dosages of 10mg, 5mg, and 2.5mg

Plant part	*Extract*	*S. paratyphi*			*S. dysenteriae*			*E. coli*			*V. cholera*		
		10 mg	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*
Leaf	ME	35 ±1	33 ±1	27.6 ±0.5	13±1	11.6 ±0.5	9.3±0.5	17 ± 1	13.6 ±0.5	11.6 ±0.5	23±1	19±1	17.3±0.57
	EA	29 ±1	26.6 ±0.5	22 ±1	14 ±1	13±1	10.3±0.5	10.6 ± 0.5	8 ± 1	8 ± 0	13±1	6.6±0.57	6.3±0.57
	CH	11.3 ±0.5	9 ±1	7.6 ±0.5	8.6±0.5	8.3±0.5	.	9.6 ± 0.5	7.6 ± 0.5	.	-	.	.
Bark	ME	21.3 ±0.5	18.6 ±0.5	13.6 ±0.5	19.3±1	13±1	11±1	18.3 ± 0.5	13 ± 1	9 ± 1	19.6±0.57	14±1	12.314±10.57
	EA	15.6 ±0.5	13 ±1	8.6 ±0.5	12±1	10.6±0.5	8±1	12.6 ± 0.5	10 ±1	8.6 ± 0.5	13.3±0.57	9±1	7.3±0.57
	CH	7.6±0.5	.	.	8.6±0.5	.	.	9 ± 1	8.6 ± 0.5	7 ± 1	6.6±0.57	6.5±0.7	.
Fruit	ME	34.6 ±0.5	31.3 ±0.5	18.3 ±0.5	14±1	11.6±0.5	9.3±0.5	16 ±1	14.3 ± 0.5	9.6 ±0.5	19±1	14.6±1.	11.6±0.57
	EA	14 ±0.5	12 ±1	10.3 ±0.5	12.3±0.5	9.6±0.5	8.3± 0.5	10.6 ± 0.5	10.3 ± 0.5	9 ± 1	9 ± 1	6.6±0.57	.
	CH	11.6±0.5	9.3±0.5	8±1	8±0	.	.	9 ± 0	8.3 ± 0.5	6.3 ± 0.5	7±1	6.5±0.7	.

Values represent mean plus standard deviations; ME: methanol extract, EA: ethyl acetate, CH: chloroform, and "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive
*Source:*The authors give data from their analysis in the form of a table

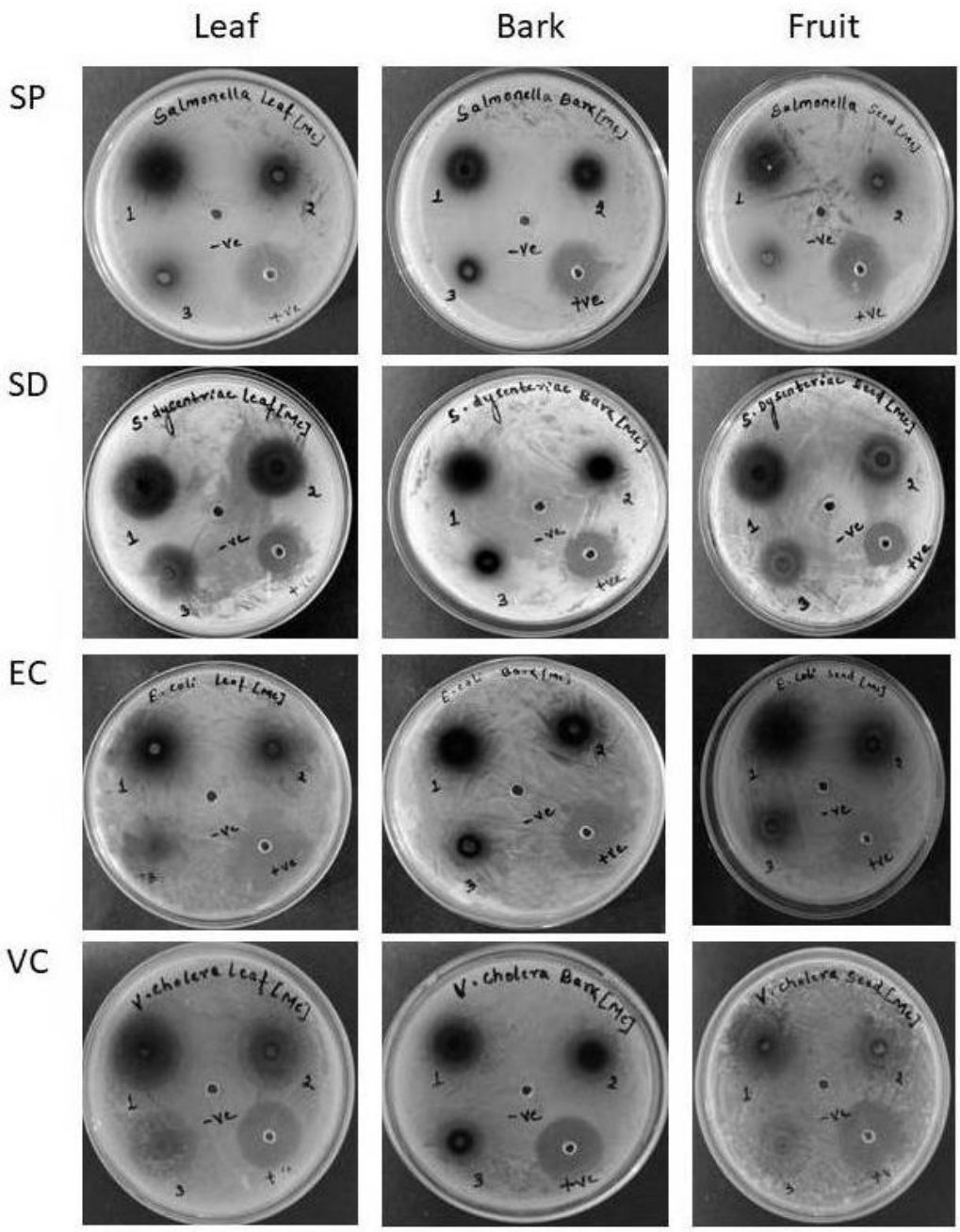

Fig. 1. Pictorial data of Antibacterial activity (Gram-negative bacteria) of Mathenol extractsof C. collinus
SP: *Salmonella paratyphi,* SD: *Shigella dysenteriae,* EC*: Escherichia coli,* VC: *Vibrio cholerae. 1: 10 mg, 2: 5 mg, 4: 2.5 mg*
Source: Authors

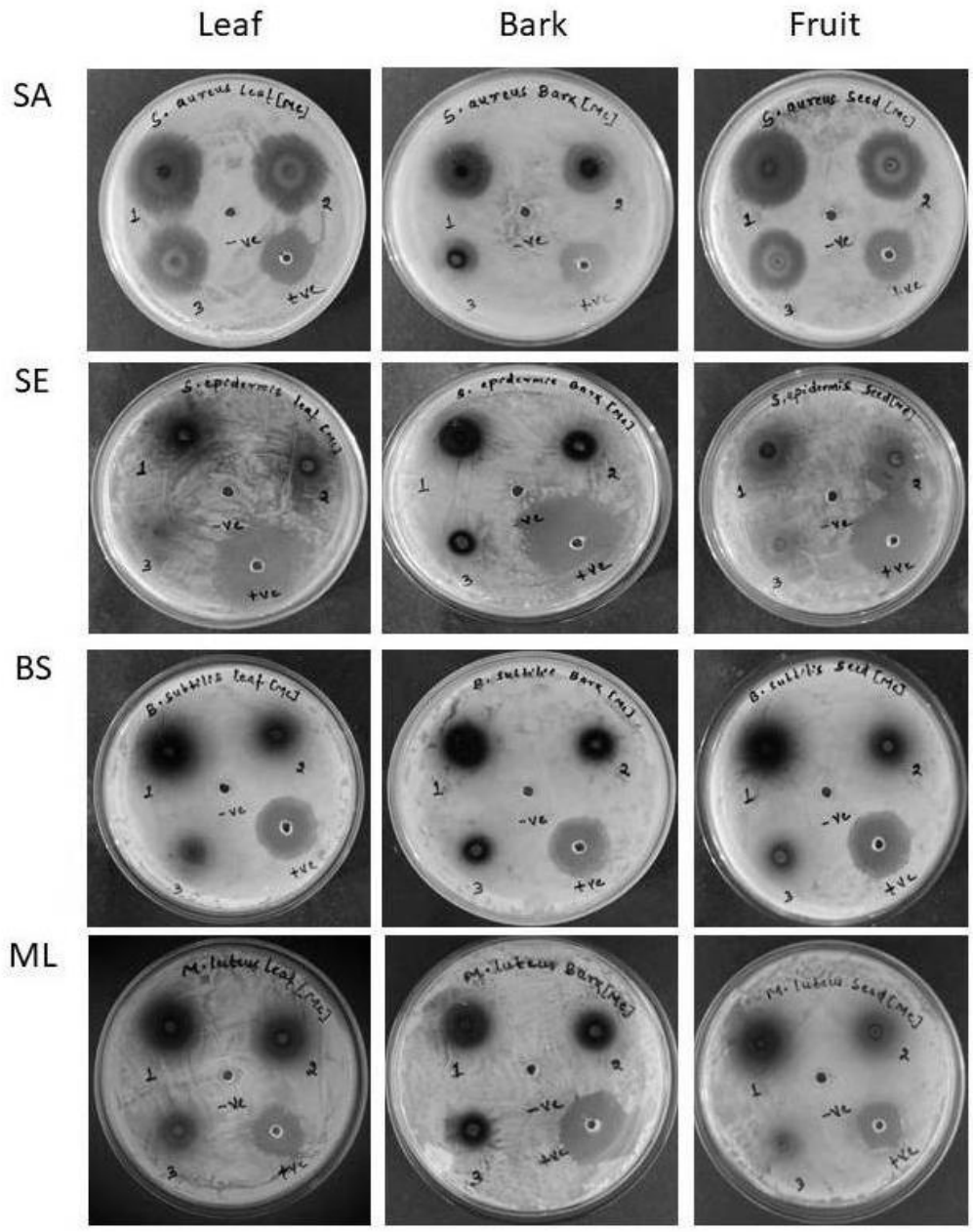

Fig. 1. Pictorial data of Antibacterial activity (Gram-positive bacteria) of Mathenol extractsof C. collinus
SA: *Staphylococcus aureus,* SE: *Streptococcus epidermidis,* BS: *Bacillus subtilis,* ML: *Micrococcus luteus,* 1: 10 mg, 2; 5 mg, 3: 2.5 mg
Source: Authors

Table 5: Antibacterial activity (% of inhibition) with respect to +' ve control

Plant part	*Extract*	*Antibacterial activity (% of inhibition) with respect to +' ve control*							
		S. paratyphi	*S. dysenteriae*	*E. coli*	*V. cholera*	*S. aureus*	*S. epidermidis*	*B. subtilis*	*M. luteus*
Leaf	Me	62.85	103	55.71	63.10	125	48.14	65.38	82.14
	EA	39.28	65	39.28	59.65	103	51.85	40.76	46.42
	CH	36.78	41.5	23.57	35.51	40.35	31.85	36.92	0
Bark	Me	48.57	65	77.14	57.24	76.07	71.48	70.38	70
	EA	30.71	46.5	52.14	44.82	55.71	44.44	48.46	47.5
	CH	27.14	0	23.57	25.17	27.14	31.85	34.61	23.57
Fruit	Me	50	85	84.28	55.17	123.5	51.85	61.53	67.85
	EA	35.71	41.5	46.42	42.41	50	45.55	40.76	32.14
	CH	33.21	40	23.57	27.58	41.52	29.62	34.61	25

ME; methanol extract, EA; ethyl acetate, CH; chloroform
*Source:*The authors give data from their analysis in the form of a table

extracts generally support earlier research that linked the existence of bioactive secondary metabolites to plants' antimicrobial properties.

According to a previous study, the ethyl acetate and methanol extracts of *C. collinus* leaf and bark were reported to suppress the growth of all four bacteria species, namely *E. coli*, *Bacillus*, *Staphylococcus*, and *Pseudomonas* (Jain et al. 2010). Similarly, Suman and Elangomathavan (2013) emphasized the antibacterial activity of hot methanol extracts against various pathogens, consistent with our observations of enhanced antibacterial efficacy in methanol extracts. Aqueous extract of *C. collinus* exhibited significant antibacterial activity against *S. typhi*, *K. pneumoniae*, *E. coli*, and *V. cholerae*, while extracts derived from hexane and ethyl acetate solvents showed significantly greater antibacterial activity, according to Ramaraj et al. (2015). Similar results were obtained by Misbah and Alok (2022), and they reported that the methanol extract from *C. collinus* leaves is effective against a variety of bacteria, including *E. coli*, *K. pneumoniae*, *L. monocytogenes*, *P. aeruginosa*, S. *typhi*, and *S. aureus*, with zones of inhibition (ZOI) varying from 18 to 30 mm.

According to a previous study by Maji et al. (2010) extracts of *C. collinus* leaf in acetone, aqueous, and benzene demonstrated profound antimicrobial activity (> 11 mm inhibition zone) against *Candida albicans, K. pneumoniae*, *Bacillus cereus*, *Vibrio cholerae*, and MDR strains of *S. aureus* and *E. coli*. The zone of inhibition of silver nanoparticles from leaves of *C. collinus* over *S. dysenteriae*, *S. aureus*, and *B. subtilis* measured as 21 ± 1, 20 ± 2, 16 ±2 mm, respectively, on Meuller Hinton Agar plates (Lourthuraj et al. 2020). *B. subtilis*, *P. aeruginosa*, and *S. aureus* are significantly inhibited by the silver nanoparticles (AgNPs) produced by *C. collinus* leaf extract (Yugal et al. 2018). The antibacterial activity of the organic extracts was considerably greater than that of the aqueous extract. This result confirms that non-polar components occur in extracts that have greater bactericidal and bacteriostatic potencies. Most of the plant-derived antibiotic compounds are presumably aromatic or saturated organic molecules that are simple to solubilize in organic solvents (Mukherjee et al. 2006).

The present investigation highlights the superior efficacy of *C. collinus* extracts in methanol and ethyl acetate, a departure from earlier findings. This variance may be attributed to the significant influence of the solvent employed in the extraction process on the efficient isolation of botanical constituents from plant material. The notable antibacterial activities observed in *C. collinus* may be attributed to specific antibacterial agents identified through GC-MS analysis in the methanol extracts of leaves, such as 4-O-Methylmannose, 1,2,3-Benzenetriol, Didodecyl phthalate, and Thiophene (Suman et al. 2013). The antibacterial screening reaffirms the traditional application of *C. collinus* extract as a therapeutic measure against various pathogenic infections, including its use as an antiseptic (Remya et al. 2018).

CONCLUSION

The overall findings of the preliminary phytochemical screening of different parts of *C. collinus* such as leaf, bark and fruit extracts (water, meth-

anol, ethyl acetate, chloroform, and petroleum ether) suggested the possibility of producing antimicrobial medicinal compounds from them in the current study. Different phytochemicals including phytosterols, glycosides, terpenoids, flavonoids, alkaloids, saponins, phenols, carbohydrates, tannins, oils, and many amino acids are the most common phytochemicals found in the extracts of *C. collinus*. Among the bacterial strains examined, methanol crude extract of leaf was shown to be efficient against *S. aureus* and moderately effective against other bacterial infections. When the antibacterial potentialities of plant extracts were compared to those of standard extracts, *S. aureus* was shown to be the most vulnerable pathogen to *C. collinus*, and *S. epidermidis* is more resistant.

This study's investigation establishes a robust scientific foundation affirming the traditional medicinal use of *Cleistanthus collinus* in the treatment of bacterial infections. The findings not only validate the efficacy of the plant in addressing bacterial pathogens but also contribute valuable insights that can inform and enhance the utilization of this botanical remedy in contemporary healthcare practices. Notably, the methanol and ethyl acetate extracts demonstrated remarkable antibacterial properties and exhibited strong antibacterial activity. The imperative for further investigation extends beyond the current study, emphasizing the necessity to isolate and identify the active principles. The chemical analysis revealed potent antimicrobial properties, emphasizing the importance of isolating pure compounds, elucidating their structures, and conducting antimicrobial tests on these components. The researchers advocate for continued studies in this domain, anticipating that such research endeavors will contribute to the development of novel antibacterial and anticancer drugs.

RECOMMENDATIONS

C. collinus leaf, bark, and fruit methanol extracts have been shown to have the most significant antibacterial activity during in vitro conditions. The preliminary phytochemical research of this medicinal plant verifies its promise by discovering a variety of secondary metabolites with antibacterial characteristics. These discoveries have provided a scientific underpinning for the plant's usage in the traditional system of medicine. Further study on this medicinal plant is recommended so that it can endure clinical testing to generate plant-based natural pharmaceuticals.

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CONFLICT OF INTEREST

The authors assert that they have no conflicting interests.

AUTHOR CONTRIBUTIONS

S. Prathamanjali conceived and designed the experiments, performed data analysis, and literature review, and took the lead in writing and finalizing the manuscript. Professor T. Vijaya, advisor provided guidance to the co-authors, overseeing the research process, and took responsibility for refining the manuscript's final version. Y. T. Rajesh Babu collaborator executed experimental procedures, contributed to data interpretation, and participated in manuscript preparation. The ultimate manuscript underwent thorough review and received unanimous approval from all contributors.

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