Phytochemical Screening and Bioactivity Evaluation of Cornus spp.

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ABSTRACT

This study evaluates the phytochemical composition and bioactive properties of plant extracts with emphasis on their antimicrobial and antioxidant potential. Crude extracts were prepared using aqueous and ethanol solvents and subjected to qualitative phytochemical screening, which revealed the presence of key bioactive compounds such as flavonoids, phenolics, tannins, saponins, and triterpenoids. Thin Layer Chromatography (TLC) analysis, coupled with DPPH bioautography, demonstrated notable antioxidant activity through visible radical scavenging bands. Antimicrobial activity assessed using the agar well diffusion method showed inhibitory effects against Escherichia coli, Staphylococcus aureus, and Saccharomyces cerevisiae. Further evaluation using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) confirmed the antibacterial efficacy of the extracts. Additionally, the extracts exhibited potential anti-biofilm activity, indicating their relevance in combating resistant microbial communities. These findings support the potential application of plant-derived phytochemicals in therapeutic and biomedical fields.

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INTRODUCTION

Phytochemicals are natural products from plants that have been widely used as medicinal and therapeutic sources. They are a promising source of bioactive compounds with strong antimicrobial properties. The presence of alkaloids, flavonoids, phenolics and terpenoids show efficacy against a wide range of pathogenic microorganisms. They have been used as traditional medicine for ages highlighting its potential to become novel drug candidates. Its uses are widely being explored in biomedical and textile fields. A good example of this is textiles when exposed to moisture, environmental conditions can carry harmful microorganism especially in health care settings. Usage of plant derived antimicrobial agents into textiles has emerged as an innovative approach to fight against proliferation of pathogens. This paper analyses the extraction of phytochemical from plants and evaluates the antimicrobial properties of them.

MATERIALS AND METHODS

Collection of the plant material

Leaves from the chosen plant species were freshly collected and immediately washed under running tap water to remove adhering dust and debris. The cleaned leaves were gently blotted dry with absorbent paper before being coarsely crushed with the aid of suitable solvents to form a paste. This initial preparation ensured maximum surface area exposure for subsequent extraction. The maceration method was employed, wherein the plant material was immersed in solvent at ambient temperature for an extended period. Occasional stirring was conducted to improve contact between the solvent and plant tissue, thereby enhancing the diffusion of bioactive compounds. This technique is particularly effective for heat-sensitive phytochemicals and is widely adopted in small-scale laboratory studies due to its simplicity and reliability.

Preparation of plant extracts

For extraction, water and ethanol were selected as solvents owing to their safety, affordability, and broad solubilizing capacity. A measured volume of 100 mL solvent was combined with 5 g of dried leaf powder. The mixture was homogenized in a mortar with continuous grinding using a pestle to facilitate release of phytoconstituents. The resulting slurry was first passed through muslin cloth to remove coarse residues, followed by filtration through standard filter paper to obtain a clear extract. The filtrate was collected in a clean beaker and allowed to evaporate naturally in shaded conditions at room temperature, ensuring gradual solvent removal without heat exposure. Once concentrated, the crude extract was transferred into an amber glass container, labelled appropriately, and stored at 4 °C to preserve its stability for further analysis.

Phytochemical analysis

The plant extracts were qualitatively checked for the presence of various phytochemical constituents by the following procedures.

Detection of Alkaloids by Iodine Test

The plant extract was treated with a few drops of iodine solution and the appearance of a blue coloration which disappears upon boiling and reappears upon cooling indicates the presence of alkaloids.

Detection of Saponins by Foam Test

A mixture of 0.5 mL of 50 mg/mL plant extract and 2 mL of distilled water was vigorously shaken for 2 minutes. The persistence of foam for at least 10 minutes indicates the presence of saponins.

Detection of Phytosterols by Salkowski's Test:

A few drops of concentrated sulfuric acid were added to the plant extract, shaken well, and allowed to stand. A red coloration in the lower layer confirms the presence of phytosterols.

Detection of Phenolic Compounds by Iodine Test:

To 1 mL of 50 mg/mL plant extract, a few drops of dilute iodine solution were added. The transient appearance of a red coloration indicates the presence of phenolic compounds.

Detection of Tannins by Braymer's Test:

To 1 mL of 50 mg/mL plant sample, 3 mL of distilled water and three drops of 10% ferric chloride solution were added. The development of a blue-green color confirms the presence of tannins.

Detection of Flavonoids by Alkaline Reagent Test:

To 1 mL of 50 mg/mL plant extract, 2 mL of 2% sodium hydroxide solution were added, followed by a few drops of dilute hydrochloric acid. A change from intense yellow coloration to colourless confirms the presence of flavonoids.

Detection of Carbohydrates by Benedict's Test:

The plant extract was treated with Benedict's reagent and gently heated. The formation of an orange-red precipitate indicates the presence of reducing sugars.

Detection of Glycosides

To the plant extract, 1 mL of distilled water and a few drops of aqueous sodium hydroxide solution were added. The appearance of a yellow coloration suggests the presence of glycosides.

Detection of Triterpenoids
To 5 mL of 50 mg/mL plant extract, 2 mL of chloroform and 3 mL of concentrated sulfuric acid were added. A grey coloration at the interface indicates the presence of triterpenoids.

Thin Layer Chromatography
Thin Layer Chromatography (TLC) is a method of analysis where the visualization of the components of a sample is reliant on the adsorption principle. The process relies upon capillary action and migration of a mobile phase on a thin silica gel surface acting as a stationary phase. In this research silica-coated paper, carrying a spot of the extract in the middle of the base line was placed in a solvent chamber. The sample travels along with the mobile phase based on molecular weight, polarity, and chemical nature. Since migration of molecules changes as a function of the chemical nature, different solvents combinations were used to qualitatively assess the compounds in the extract. Bioautographic examination on the chromatogram would highlight about the bioactive molecule in the extract. For better visualisation, the TLC plate is exposed to Iodine vapours, which facilitates a better analysis for unsaturated organic compounds noted by dark bands. The Rf value is calculated as a ratio of the distance travelled by the solute to the distance travelled by the solvent.

In-Vitro antioxidant screening by TLC bioautography assay
TLC bioautography assay is a chemical analysis technique for determining the invitro antioxidant activity of bioactive compounds or plant extracts via a combination of DPPH (2,2-diphenyl-1-picrylhydrazyl) chemical detection and thin-layer chromatography. The process applies the high-speed chemical screening property of TLC followed by direct detection of antioxidant activity on the chromatogram plate. After chromatographic separation of extract fractions on a silica-coated plate, air-dried plate is sprayed with DPPH solution, which high lights as a pale-yellow band to deep violet free radical background with high absorbance at 520 nm. Upon reaction with an antioxidant (radical scavenger), DPPH gets reduced to a colour change to pale yellow or colourless. This color shift is observed on the TLC plate as yellow spots against purple, indicating the position and existence of antioxidant molecules in the extract. This method allows for visible detection of bioactive molecules and presents a rapid, low-cost method of screening for antioxidant activity with chemical and biological information of plant extracts.

Antibacterial and antifungal activity
The antibacterial and antifungal activity of the crude plant extracts was tested by agar well diffusion method on the gram-negative Escherichia coli, gram-positive Staphylococcus aureus, and Saccharomyces cerevisiae, respectively. The bacterial and fungal cultures were swabbed onto the Mueller Hinton agar plates, and the plant extract was loaded into wells. The antimicrobial activity of the plant was measured by the

presence and size of the zone of inhibition around the sample after incubation for 24hr. Oxacillin was used as the positive control.

Minimal Inhibitory Concentration and Minimum Bactericidal Concentration

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antibacterial agent expressed in µL which, under strictly controlled in vitro conditions, completely prevents visible growth of the test strain of an organism. Minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobial that will prevent the growth of an organisms after subculture onto antibiotic-free media. The MBC test is an extension of MIC assay and helps differentiate the bactericidal effect from bacteriostatic and inhibitory effects. MBC indicates complete bacterial death.

Biofilm Inhibition Assay

Biofilms are structured populations of bacteria autogenerating a polymeric extracellular matrix, evolved to protect the organisms from unfavourable environmental situations. Biofilms are also highly implicated in chronic antibiotic-resistant infections due to the protective nature of the biofilms. This led the group to investigate the activity of the extract against biofilms. If the results yielded were positive then it would be of great worth, as inhibition of biofilm would make it easier to target individual bacteria.

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical analysis was evaluated for the ethanol and aqueous extracts, and it was observed that phenols were present in the aqueous extracts and triterpenoids were present in the ethanolic extracts

SN	PHYTOCHEMICAL	ETHANOL EXTRACT	AQUEOUS EXTRACT
1	Alkaloid	Absent	Absent
2	Saponins	Absent	Absent
3	Phytosterols	Absent	Absent
4	Phenols	Absent	Present
5	Tannins	Absent	Absent
6	Flavonoids	Absent	Absent
7	Carbohydrates	Absent	Absent
8	Glycosides	Absent	Absent
9	Triterpenoids	Present	Absent

Table 1. Phytochemical analysis of the plant extracts

Thin Layer Chromatography

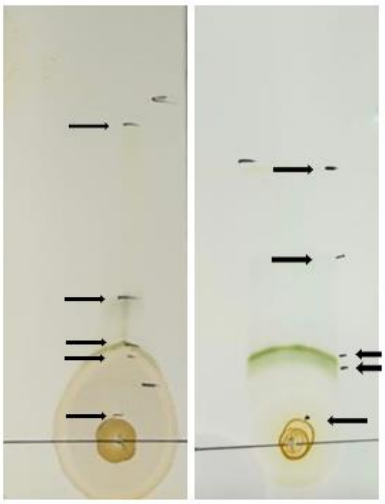
The Thin Layer Chromatography (TLC) of ethanol and aqueous extracts revealed a broad range of bioactive compounds in the sense of the number of bands and respective Rf values in different mobile phases. In ethanol extract, when ethanol was used as the mobile phase, four bands were observed with Rf values of 0.74, 0.86, 0.80, and 0.71. These relatively high values for Rf are characteristic of the occurrence of medium polarity compounds. They are flavonoids, phenolic acids, or terpenoids, and they are soluble in polar protic solvents. One band at 0.80 Rf occurs in methanol, indicating the domination of a specific compound with medium polarity, a straight phenol or flavone. Non-polar mobile phase chloroform exhibited five various bands (Rf values 0.96, 0.64, 0.30, 0.26, and 0.10), indicating a broad array of polarities of compounds in the ethanol extract. High Rf values (0.96 and 0.64) indicate the occurrence of non-polar terpenes or alkylated flavonoids, whereas low values indicate more polar aromatic compounds, i.e., glycosides or tannins. No bands were detected when water was used, reflecting the absence of very polar hydrophilic compounds in the ethanol extract. Three bands with Rf values 0.74, 0.61, and 0.55 were detected from the aqueous extract on using ethanol as a mobile phase, which reflects the presence of moderately polar compounds like saponins, tannins, or phenolic acids. Methanol exhibited two bands with Rf values of 0.84 and 0.21, representing the composite presence of non-polar molecules and strongly polar constituents like sugar-bound flavonoids. Non-polar chloroform contained five bands (Rf values: 0.92, 0.41, 0.29, 0.18, 0.07), which once again indicated the existence of a wide range of compounds in the aqueous extract. Remarkable is the Rf value of 0.92, indicating the existence of some unwanted nonpolar compounds, possibly extracted

owing to solvent interactions or breakdown of larger polar molecules during processing. The lower Rf values are more polar bioactive constituents that were still mobile by interaction with chloroform. Just like the ethanol extract, no separation of the aqueous extract was achieved when water alone was used as the mobile phase, once more indicating the inefficiency of water alone in mobilizing compounds on silica plates. As a whole, the TLC profile of each extract captures the chemical diversity of phytochemicals, and ethanol and aqueous extracts both capture the presence of compounds along a gradient of polarities. The variation in the Rf values of mobile phases suggests the importance of selection of solvent to reveal the total phytochemical profile of natural extracts and concludes that there are some important classes of bioactive compounds such as flavonoids, tannins, phenolic acids, terpenes, and glycosides responsible for the perceived bioactivities of the plant in other assays.

Sample	Mobile Phase	Number of Bands	Rf Value
Ethanol Extract	Ethanol	4	a) 0.74 b) 0.86 c) 0.80 d) 0.71
	Methanol	1	a) 0.80
	Chloroform	5	a) 0.96 b) 0.64 c) 0.30 d) 0.26 e) 0.10
	Water	0	-
Aqueous extract	Ethanol	3	a) 0.74 b) 0.61 c) 0.55
	Methanol	2	a) 0.84 b) 0.21
	Chloroform	5	a) 0.92 b) 0.41 c) 0.29 d) 0.18 e) 0.07
	Water	0	-

Table 2. Thin layer chromatography on different mobile phase

Fig 1. Thin Layer Chromatogram of the extracts



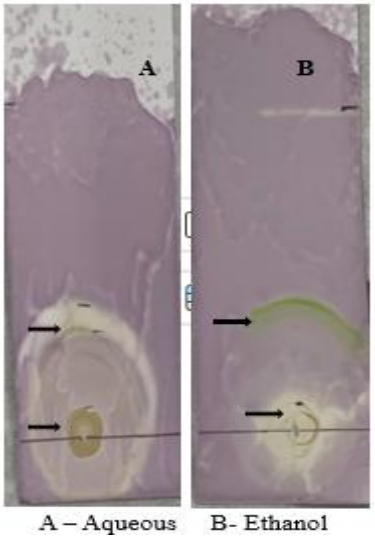
A – aqueous B- Ethanol

In-Vitro antioxidant screening by TLC bioautography assay

DPPH bioautography analysis of the aqueous and ethanol extracts revealed antioxidant compounds as yellow spots on the purple background after spraying with DPPH. A single antioxidant compound was detected according to the ethanol, methanol, and chloroform mobile phases in the ethanol extract with Rf values of 0.87, 0.86, and 0.10, respectively. The relatively high Rf values in methanol and ethanol suggest that the antioxidant compounds are moderately polar and are likely such classes as flavonoids or phenolic acids, which have been shown to be radical scavengers. The low Rf value of 0.10 in chloroform suggests a more polar antioxidant but one that still had some movement on the TLC plate, possibly a tannin or polyphenol. There were no antioxidant compounds seen using water as the mobile phase, and this indicates that water is not capable of mobilizing or separating the antioxidant components on silica. The aqueous extract was seen to have a broader spread of antioxidant activity with many yellow bands visible. The existence of strongly antioxidant, moderately polar substances in the extract was attested by ethanol and methanol spots with Rf values of 0.84 (EtOH) and 0.95 and 0.82 (MeOH). The obtained values are close to those provided by the ethanol extract and confirm the hypothesis that the two extracts contain the same antioxidant components such as flavonoids or phenolic derivatives. With the use of chloroform as the mobile phase, two antioxidant spots appeared at Rf values of 0.40 and 0.08, indicating that despite a non-polar system, the aqueous extract does contain some antioxidant compounds with varying polarities, perhaps alkaloids or glycosidic phenols. Similar. Water as a mobile phase also did not produce any visible antioxidant bands, due perhaps to its inability to induce complexity migration on the TLC silica plate. In general, DPPH bioautography assay results suggest ethanol and water extracts containing

antioxidant compounds, but the water extract contained more antioxidant-active spots with reference to diversity and number. Rf values are those of fairly polar phytochemicals, which means the majority of antioxidant activity would be attributed to the presence of phytochemicals such as flavonoids, polyphenols, and tannins. The effectiveness of ethanol and methanol as the mobile phases shows that they work well in the efficient separation of antioxidant components of plant extracts.

Fig 2. TLC Bioautography Assay of antioxidant activity



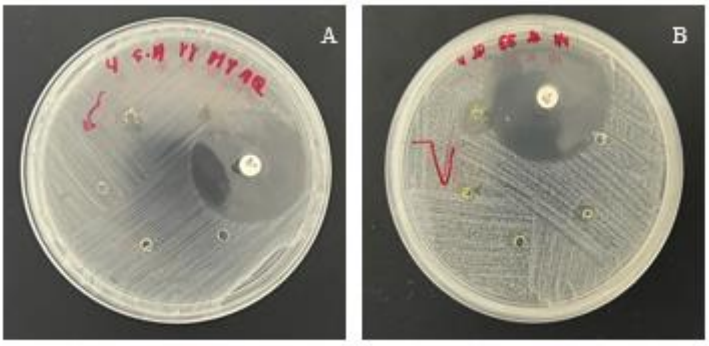
Antibacterial and antifungal activity

The antimicrobial activity of the plant extract was evaluated for various working concentrations and compared with a positive control which showed a zone of clearance in Staphylococcus aureus plate, of diameter 35mm with aqueous extract and 38mm with ethanol extract. Well demarcated zone of clearance of 10mm was obtained in both ethanol and aqueous extracts in Staphylococcus aureus. No growth was recorded in plates containing Escherichia coli. This indicated the presence of antibacterial activity against gram positive bacteria at the highest concentration of extract tested.

Extract	Zone of inhibition (mm)								
	Staphylococcus aureus					Escherichia coli			
	Positive control	2.5 µL	5.0 µL	7.5 µL	10 µL	2.5 µL	5.0 µL	7.5 µL	10 µL
Aqueous	35	-	-	-	10	-	-	-	-
Ethanol	38	-	-	-	10	-	-	-	-

Table 3. Antibacterial Activity

Fig 3: Antimicrobial activity of the aqueous and ethanol extract on S.aureus



A – Aqueous B - Ethanol

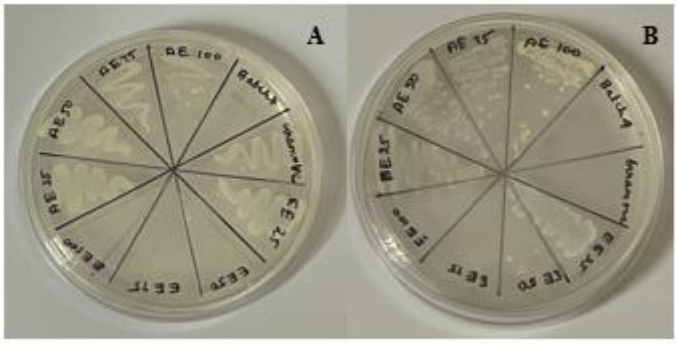
Minimal Inhibitory Concentration and Minimum Bactericidal Concentration

Inhibition of bacterial growth was found in ethanol extract with E. coli at a concentration of 100µL. However, the presence of bacterial growth in aqueous extract with both S. aureus and E. coli indicated that there was no bacterial growth inhibition. This implied that the concentrations of extract chosen for testing proved to have no effect and further testing using higher concentration is required. This is in accordance with the results of MBC, where there is bacterial growth in aqueous extract plates in both E. coli and S. aureus and in ethanol extract at lower concentrations, which shows the lack of bactericidal activity of the extracts and the selected testing concentrations.

	Staphylococcus aureus				Escherichia coli			
Aq. Extract	2.5µL	5.0µL	7.5µL	10.0µL	2.5µL	5.0µL	7.5µL	10.0µL
	++	++	++	+	++	++	++	+
EtOH Extract	2.5µL	5.0µL	7.5µL	10.0µL	2.5µL	5.0µL	7.5µL	10.0µL
	++	+	NG	NG	++	+	NG	NG

Table 4. Minimal Inhibitory Concentration

Fig 3 Minimum Bactericidal Concentration on S.aureus



A – Aqueous B - Ethanol

Biofilm Inhibition Assay

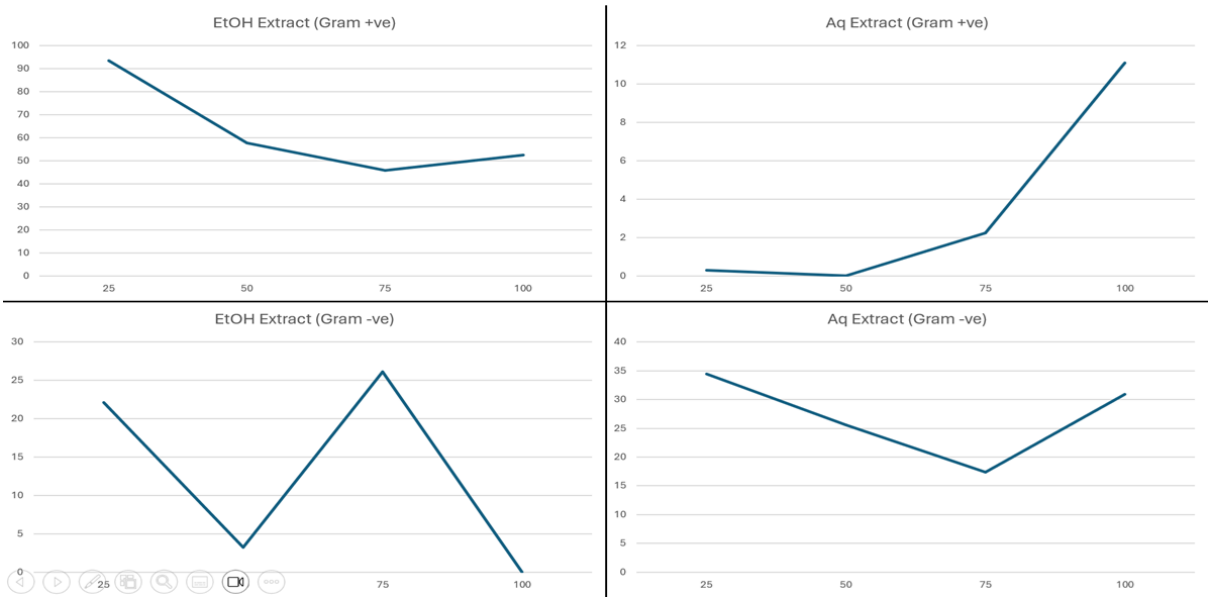
The biofilm inhibition test by aqueous (Aq. E) and ethanolic (E. E) plant extracts against Escherichia coli and Staphylococcus aureus shows a clear distinction in antimicrobial activity by both the nature of the extract and the bacterial strain. In the presence of aqueous extract, Escherichia coli caused a remarkable and repeatable reduction in the biofilm production at every concentration attempted, in a dose-response manner in general. Inhibition was 34.4% at 25 µL, climbed to a maximum of 25.62% at 50 µL, and persisted at extremely high levels at 75 µL (17.37%) and 100 µL (30.9%). The implication is that the aqueous extract is loaded with hydrophilic molecules which can penetrate and damage the biofilm matrix of this Gram-negative bacterium and thus exert potent inhibition at comparatively low concentrations. Staphylococcus aureus was less susceptible to the same extract. At 25 µL, inhibition was effectively zero (0.3%), no response (NR) at 50 µL, partial inhibition at 75 µL (2.25%) and 100 µL (11.1%). Difference indicates that S. aureus potent inhibitory compounds are not present in the aqueous extract, or that they may be unstable, or poorly absorbed by Gram-positive bacteria under these experimental conditions. Ethanolic extract yielded results that were the opposite. In the case of Staphylococcus aureus, the extract was most inhibitory

against biofilm in the 25 µL (93.37%) and was equally active in a manner of increasing concentrations: 57.83% in 50 µL and 45.93% in 75 µL, and 52.4% in 100 µL. This trend shows the efficacy of lipophilic or semi-polar phytochemicals within the ethanolic extract that could be highly active against Gram-positive bacterial structures, especially those of S. aureus, with a peptidoglycan cell wall, which is tough and could be more vulnerable to such chemicals. The same ethanolic extract was significantly weaker against E. coli. At 25 µL, it was 22.1%, fell dramatically to 3.27% at 50 µL, increased again to 26.11% at 75 µL, but failed to react at 100 µL. This non-linear pattern not only suggests minimal efficacy of ethanolic components against Gram-negative biofilms but also suggests a nonlinear, inhibitory, or saturating effect of the constituents on E. coli biofilm processes.

Plant extracts	Percentage Biofilm Inhibition of extracts (%)							
	Staphylococcus aureus				Escherichia coli			
	2.5µL	5.0µL	7.5µL	10.0µL	2.5µL	5.0µL	7.5µL	10.0µL
Aqueous	0.3	NR	2.25	11.1	34.4	25.62	17.37	30.9
Ethanol	93.37	57.83	45.93	52.4	22.1	3.27	26.11	NR

Table 5. Biofilm Inhibition

Fig 5. Percentage Biofilm inhibition of Aqueous and ethanol extract in E.coli and S.aureus



The biofilm inhibition profiles clearly indicate the role of the type of solvent system utilized in extraction as a key determinant for the plant compound's antimicrobial nature. The aqueous extract with polar compounds exhibited specific inhibition of E.coli, a Gram-negative bacterium, likely due to enhanced penetration or site-directed inhibition of the biofilm-associated processes. On the other hand, the ethanolic extract, which was rich in polar or moderately polar phytochemicals, was extremely inhibitory towards S. aureus, possibly through disruption of membrane integrity or blocking surface adhesion proteins that are crucial for biofilm formation in Gram positive bacteria. These results are helpful in guiding further purification and characterization of bioactive compounds and in controlling antibiofilm strategies as a function of bacteria type and solvent polarity, a critical step toward the creation of plant-derived antimicrobial therapies.

CONCLUSION

The results from the nonspecific qualitative phytochemical analysis of the crude plant extracts can be used to further study in the medicinal and therapeutic uses of Cornus spp. The presence of phenols detected in aqueous extract further confirms the findings of antibacterial and antioxidant properties and the presence of triterpenoids in the ethanol extract, support the plant's potential therapeutic properties and uses in herbal based medicine. This study can contribute to more comprehensive research into growing evidence that suggests the widespread use of natural herbal extracts in drug discovery and development.

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