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Phytochemical Screening and Antimicrobial Activity of Methanolic Extract from Genus Phellodendron (Cork Tree) Barks

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Abstract

The present study examined the phytochemical and antimicrobial properties of a methanolic bark extract from a cork tree (Genus Phellodendron). Alkaloids, carbohydrates, steroids, reducing sugars, oils and fats, gums, volatile oil, flavonoids, proteins, amino acids, cysteine, anthraquinone glycoside, tannins, and phenolic chemicals were all found during the phytochemical screening. Due to the presence of oils, non-reducing polysaccharides and saponin glycoside were not present, and the solubility test verified insolubility in 90% ethanol and water. Studies on phytochemicals and antimicrobials were conducted using 95% methanolic extracts. The four test organisms employed in antibiotic investigations were staphylococcus aureus, Escherichia coli, Enterococcus species, and Pseudomonas auriginosa. Two techniques were used to experiment: the disc-diffusion method and the cup-plate approach.Given that the methanolic extract's Minimal Inhibitory Concentrations (MIC) were roughly 256 µg/ml, it is clear that cork trees have antimicrobial properties. Their mode of action may involve blocking the synthesis of proteins at the transcriptional or translational level or peptidoglycan synthesis at the membrane level. The presence of marmine (immature bark) and fagarine (mature bark), which also have antiulcer and abortifacient properties, may be the cause of the bark extract's antibacterial qualities. The findings offer encouraging baseline data for the possible application of this plant and some of its components in managing microbial illnesses.

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INTRODUCTION

Cork trees (genus Phellodendron) are a group of rue family (Rutaceae) found in eastern Asia and typically have bark resembling cork. The Amur, or Japanese, cork tree (Phellodendron amurense) can withstand various growth situations and is useful as a lawn and shade tree [1]. Deciduous cork trees have dark green, pinnately split leaves that turn yellow in the autumn. Summer brings little greenish-yellow blossoms. Small clusters of black drupes like grapes are produced by female

trees, which attract birds and other creatures. The cork oak (Quercus suber), an evergreen oak tree endemic to the Mediterranean region, is known for its outer bark [2]. Cork oaks are widely distributed in North Africa, Portugal, Spain, and southern France and Italy. With glossy green, holly-like leaves and a large, round-topped head, the tree typically stands around 18 meters (60 feet) tall. Once the rough outer bark has been removed, the inner bark forms a new outer sheath of bark from which cork is extracted. After that, the outer sheath can be removed and reformed [3]. The outer bark, often known as cork, shields the tree from the heat and dry winds of the Mediterranean summer and is not as crucial to its survival as the inner bark. Because the inner bark of the cork oak grows remarkably homogeneous and continuously regenerating tissue, it is possible to peel the cork repeatedly. [4] This tissue produces enough cork cells to the exterior after the outer bark has been peeled off such that, in a healthy tree, a uniform new cork sheathing of 2.5 to 5 cm (1 to 2 inches) emerges in 3 to 10 years. Commercial cork slabs are produced by removing this regenerated layer. [5] The typical lifespan of a cork oak is 150 years. For the first 20 years of its life, the tree produces very little cork, and the bark that is harvested at the first stripping (about age 25) is rough, irregular, and of little commercial use. [6] It has been demonstrated that methanolic extracts of maize and cork oak leaves are efficient against Culiseta annulata mosquito larvae. Methanolic preparations of cork oak bark have shown efficacy in combating Candida albicans-caused fungal infections.

MATERIALS AND METHODS:

Phellodendron stem bark was gathered at the Indian Botanical Garden in India. After being washed with water, the stem bark fragments were left to sundry for five days [7]. Methanol (200 ml, 72 hrs) was used to extract the 500 g of air-dried and powdered stem bark at room temperature, yielding a crude extract (53.8 g). [8] The extract was kept at 8° C in a refrigerator. The extract smells good and is light chocolate brown. 75.2% of the cork tree's methanolic extract was produced. [9] Various concentrations of 100 mg, 50 mg, 25 mg, 10 mg, 100 µg, 50 µg, and 25 µg per milliliter of methanol were used to reconstitute the crude extract. Four clinical bacterial strains—Escherichia coli, Staphylococcus aureus, Pseudomonas auriginosa, and Enterococcus sp.—were used as test organisms. Among these were two gram-negative bacteria (Escherichia coli and Pseudomonas auriginosa) and two gram-positive bacteria (Enterococcus sp. and Staphylococcus aureus). [11] The test organisms were further identified using accepted techniques, and their morphological and biochemical traits were verified. The bacterial strains were kept on nutrient agar and cultivated at 37 °C in an incubator. [12] Every microbe was kept in its plate or broth suspension at four °C. To identify the constituents (alkaloids, carbohydrates, steroids, reducing sugar, fats, oils, gums, volatile oil, flavonoids, proteins, amino acid, anthraquinone, glycoside, tannins, and phenolic compound), phytochemical tests were performed using the methanolic extract of Cork tree bark using standard procedures. [13] The Bauer-Kirby disc diffusion method and the cup plate method were the foundation for the antibiotic sensitivity test.

RESULTS AND DISCUSSION

Screening using phytochemicals Alkaloid, carbohydrate, steroid, reducing sugar, gums, volatile oil, flavonoids, protein, amino acid, cysteine, anthraquinone glycoside, tannins, and phenolic compounds are among the medicinally active ingredients that were found through phytochemical screening. **Table 1** presents the details of this investigation.

Given the existence of these metabolites, the plant may have significant phytomedical value. Geographical regions, soil makeup, and plant age all contribute to inequalities. Along with anthraquinones, flavonoids may be the reason for the plant's usage as an analgesic, inflammatory aprain, and antioxidant. The presence of alkaloids and tannins in the plant extract indicates that it possesses antibacterial properties. Enzymes, cell membrane transport proteins, microbial adhesions, and other substances can all be inactive by tannins. Through substrate and metal ion deprivation, tannins also prevent the growth of bacteria because of their capacity to attach to proteins and metals. The presence of tannins may also indicate that it has antiparasitic and wound-healing properties as an astringent. Phenol exhibits antiviral and antioxidant properties.

The redox characteristics of phenolic compounds, which can be crucial in squelching singlet and

Table 1 Information of the cork tree methanolic extract phytochemical test

Experiment	Observation
Alkaloid test	
a) Mayer's reagent	Precipitate observed
b) Wagner's reagent	A reddish-brown precipitate was observed.
Carbohydrate test	
a)Molish test	A Violet ring appeared.
Non-reducing polysaccharides test	
a)Iodine test	No blue color appeared.
Steroid test	
a) Salkowski reaction	The chloroform layer appeared, and the acid layer showed greenish-yellow fluorescence.
Reducing sugar test	
a) Fehling's test	First, a yellow and then a brick red color precipitate.
Saponin glycoside test	
a)Foam test	No foaming.
Test for fats and oils	Filter paper gets permanently stained with oil.
Solubility test	Insoluble in 90% ethanol and water.
Test for gums	Fehling test red color appeared.
Test for volatile oil Sudan's abolition added to a thin section of drug	Globules obtain the red color.
Flavonoid test Residue+lead acetate solution	A yellow color appeared.
Protein test	
a)Biuret test	A violet color appeared.
Amino acid test	
a)Ninhydrin test	A purple color appeared.
Test for cysteine	The black precipitate of lead sulfate formed.
Test for anthraquinone glycoside	
a) Borntrager's test	The ammonical layer turned pink.
Test for tannins and phenolic compounds	
a) Lead acetate solution	White precipitate.
b)Dilute iodine solution	Red color solution.

triplet oxygen species, breaking down peroxide, and neutralizing free radicals, are the primary cause of their antioxidant action. Terpenes indicated that it possesses antiviral and antitumor properties. It can be employed in natural body cosmetics and fragrances, according to the presence of volatile oil. The oil's ability to treat skin infections topically makes this even more crucial. Bacterial infections can be treated with the oil since it affects the test organism.

ANTIMICROBIAL ACTIVITY

A 95% methanolic extract of the cork tree's stem bark was tested for antibacterial properties. It was discovered that the extract had activity against Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus sp., and Escherichia coli. Neither the negative nor the positive controls showed any growth. The findings of the antibacterial activity using the cup plate method (**Figure 2**) and the agar disc diffusion method (**Figure 1**) are shown in **Table 2** and **Table 3**. Disc diffusion method Inhibition Distance (ID) varied from 0 to 19 mm. The ID ranged between 0 and 9 mm for gram-positive bacteria (Staphylococcus aureus and Enterococcus sp.) and 0 to 10 mm for gram harmful bacteria (Escherichia coli, Pseudomonas aeruginosa). Escherichia coli (ID = 10 mm) was the most sensitive gram-negative, and Staphylococcus aureus (ID = 9 mm) was the most sensitive gram-

Table 2 Inhibition results for the disc diffusion method obtained against four test species at varying extract concentrations

Organisms	Standard Gentamycin (10mg)	Different concentrations of Cork tree extract						
		25µg/ml	50 µg / ml	100 µg /ml	10mg /ml	25mg/ml	50mg/ml	100 mg/ml
		Inhibition Distance (ID)						
Staphylococcus aureus	19 mm	0.8 mm	0.8 mm	0.7 mm	9 mm	0.8 mm	0.8 mm	0.9 mm
Escherichia coli	22 mm	0.7 mm	10 mm	0.5 mm	8 mm	0.6 mm	0.8 mm	10
Enterococcus sp	21 mm	0.5 mm	0.7 mm	0.0 mm	0.0 mm	-	-	-
Pseudomonas aeruginosa	23 mm	0.65 mm	0.6 mm	0.7 mm	0.0 mm	-	-	-

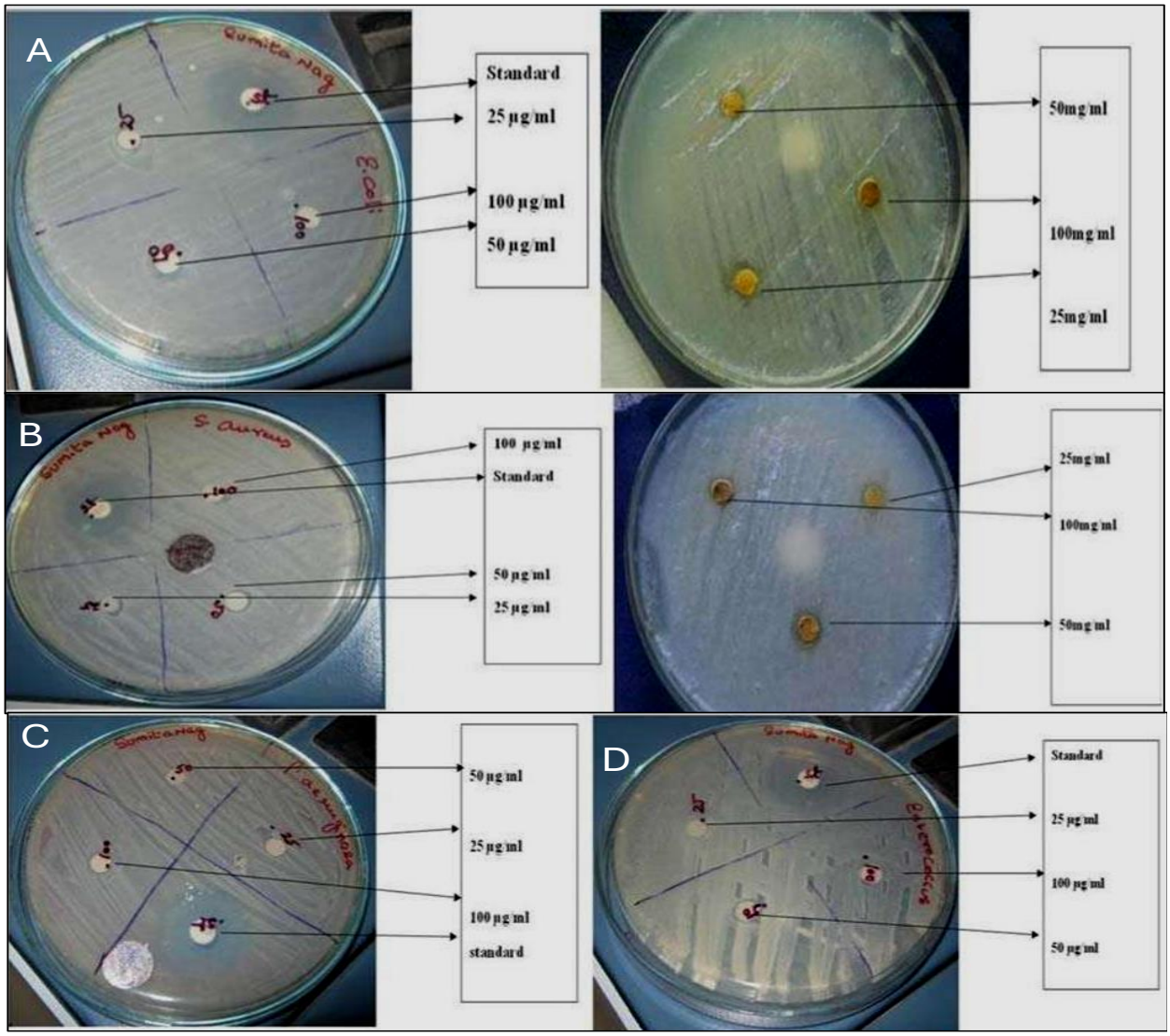

Figure 1 a)Escherichia Coli b) Staphylococcus Aureus c) Pseudomonas Aeruginosa d) Enterococcus sp.

positive. For Enterococcus species and Pseudomonas aeruginosa, no action was seen at 10 mg/ml. The standard reference antibiotic, Gentamycin ID up to 15 mm, has more vital values than these. In the cup plate method, the extract demonstrated notable inhibitory action against the two test species, Staphylococcus aureus and Escherichia coli.

The Minimum Inhibitory Concentration (MIC) for the methanolic extract ranged from 25 µg/ml to 100 mg/ml for all test species, inhibiting all tested bacteria. Previous investigations have indicated that the methanolic extract's MIC is approximately 256 µg/ml (Sharma et al., 2011). In the whole MIC (25, 50, 100 µg/ml and 10, 25, 50, 100 mg/ml) collected for both techniques, Escherichia coli and Staphylococcus aureus were more sensitive than Enterococcus and Pseudomonas aeruginosa. The significant action on Staphylococcus aureus validates the highest activity seen in solid media, indicating that this organism is among the most vulnerable. The methanolic extract had less of an inhibitory effect on the bacterial strains than the antibiotic standard Gentamycin. The ID ranged from 0.0 to 19 mm for every tested bacterium in the crude extract. The cork tree's bark methanolic extract demonstrated both bactericidal and bacteriostatic properties in this investigation, similar to those of Marmonier plant extract.

The antibacterial activity of the bark's methanolic extract was tested on test species, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Enterococcus sp. However, the varying degrees of antimicrobial properties of the crude extracts and their fraction concentrations may be explained by differences in their chemical composition. Additionally, the genetic content of plasmids that are easily transferred between strains and the composition of cell walls may explain the variations in susceptibility. According to the MIC values, the most vulnerable organisms to the extract were Escherichia coli and Staphylococcus aureus. Additionally, the data indicate a similar susceptibility of gram-positive and gram-negative microorganisms. This may suggest that the extract's mode of action was unrelated to the makeup of the cell wall. In general, it is challenging

Table 3 Values of inhibition against two test species at varying extract concentrations for the cup-plate method

Organisms	**Different concentrations of Cork tree extract**		
	25 mg/ml	50 mg/ml	100 mg/ml
	Inhibition Distance (ID)		
Staphylococcus aureus	19 mm	17 mm	13 mm
Escherichia coli	13 mm	15 mm	14 mm

Values of inhibition against two test species at varying extract concentrations for the cup-plate

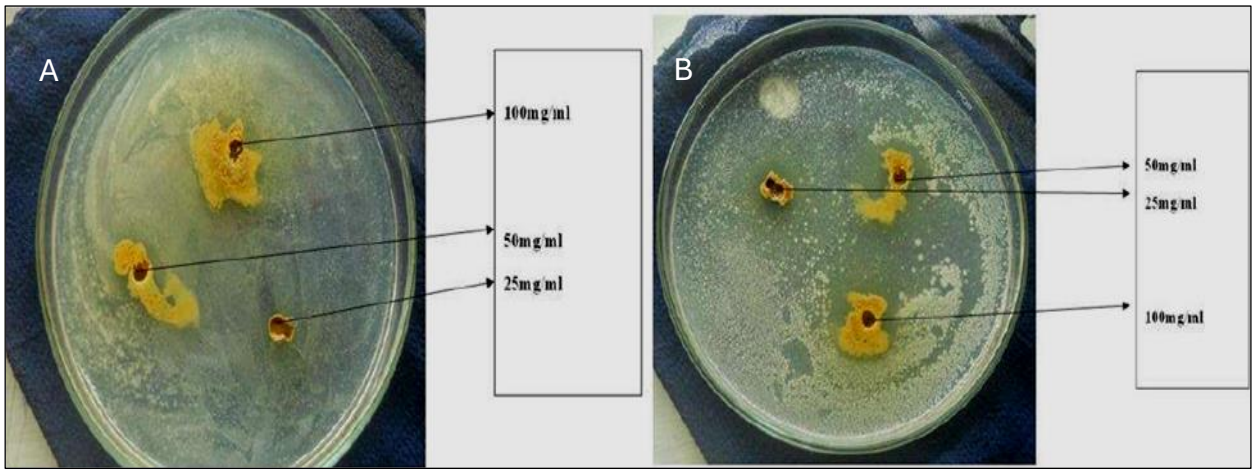

Figure 2 Inhibition of organisms using varying extract concentrations for the cup-plate method a) Escherichia Coli b) Staphylococcus Aureus

to assign the activities seen to a group of chemical compounds while viewing the results of phytochemical screening.

The study's conclusion demonstrated the bark extract's potency. This implies that the cork tree's bark may contain harmful principles. These findings offer encouraging baseline data for the plant's possible application in managing bacterial illnesses, and its antibacterial qualities may serve as the foundation for creating novel antibacterial agents with a wide range of activity.

CONCLUSION

This study makes it clear that the cork tree's bark methanolic extract contains a variety of phytoconstituents that indicate its potential applications in a range of antimicrobial medicinal applications. Because the extract has antibacterial action against the four test organisms employed here, the plant's bark can be utilized to treat a variety of infectious diseases in humans brought on by microbes. The plant may be used in the future for additional study on the creation of antibacterial phytomedicines, according to the presence of a range of secondary metabolites and the extract's effects on the microbe.

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