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Research Article

Antimicrobial and phytochemical profiling of *nicotiana tabacum* leaf extract: a potential source of bioactive compounds

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ABSTRACT

Tobacco (Nicotiana tabacum) leaves are traditionally valued as therapeutic agents in various Asian countries, including Bangladesh, due to their diverse bioactivities, including antimicrobial, antifungal, anthelmintic, and anti-Alzheimer's properties. The aim of this study was to evaluate the antimicrobial and phytochemical properties of Nicotiana tabacum leaf extracts. Alcoholic extracts were prepared using 95% ethanol, 95% methanol, and water in a 1:3 ratio for comparative analysis. Antimicrobial activity was assessed using the agar cup diffusion method against a range of bacterial and fungal strains, and phytochemical screening for alcoholic extracts was carried out following some standard methods. The methanol extract demonstrated significant antimicrobial activity, producing inhibition zones of 20.0 mm, 25.0 mm, and 32.0 mm against Streptococcus agalactiae at extract concentrations of 50 µL, 100 µL, and 150 µL, respectively. Additionally, it showed inhibition against Candida albicans, with inhibition zones of 15 mm, 25 mm, and 30 mm, alongside other Candida species. The water extract exhibited a high inhibition zone of 47.0 mm against Escherichia coli at 150 µL, along with substantial antifungal activity. Ethanol extracts demonstrated similar efficacy, with inhibition zones up to 39.0 mm against Escherichia coli and Candida species. The results highlight the potential of Nicotiana tabacum leaves as a source of bioactive compounds with antimicrobial properties, warranting further exploration for therapeutic applications.

Keywords: Tobacco leaf extract, antimicrobial activity, phytochemical composition, agar cup diffusion, alternative therapeutics

Introduction

The rise of multidrug-resistant pathogens has intensified the need for new antimicrobial agents derived from natural sources. Plants have long been recognized for their therapeutic properties, with specific species yielding bioactive compounds that demonstrate antimicrobial, anti-inflammatory, and antioxidant properties. Tobacco is traditionally known for its use in tobacco products, but recent studies revealed its potential as a source of valuable phytochemicals with diverse bioactivities (Zhang *et al.*, 2024).

Nicotiana tabacum leaves contain various phytochemicals, including alkaloids, phenolic compounds, and flavonoids, which are associated with a range of pharmacological effects (Zhang *et al*.,2024 and Ameya *et al*., 2017). The primary alkaloid, nicotine, alongside other secondary metabolites, has been shown to possess antimicrobial properties, offering a basis for exploring these extracts as alternatives or complements to conventional antibiotics (Rawat *et al*.,2013). Given the increasing focus on natural products in drug discovery, the study of tobacco's bioactive components represents a promising avenue for developing novel antimicrobial agents.

Moreover, phytochemical analyses demonstrated that *N. tabacum* leaf extracts contain significant levels of antioxidants, which could contribute to their therapeutic effects by mitigating oxidative stress—a key factor in the pathogenesis of many chronic diseases (Stéphane *et al.,* 2021).The aim of this current study was to assess the antimicrobial efficacy and phytochemical profiling of *N. tabacum* leaf extract, thereby contributing to research on plant-based bioactive compounds and their applications in pharmacology.

Materials and methods

Sample collection

Fresh leaves of tobacco were purchased from Enayetpur Market in the Sirajganj district of Bangladesh and authenticated by the Head of the Department of Microbiology at Khwaja Yunus Ali University, Enayetpur, Sirajganj. The leaves were washed with tap water to remove contaminants and extraneous material. Next, the leaves were chopped into small pieces and sun-dried for seven days. To ensure complete drying, the leaves were further dried in an oven at 120° C for 2 hours and then ground into a fine powder using a blender. Then the powdered leaves were stored in an airtight container at 4° C until further analysis (Singh et al., 2022, and Stéphane et al., 2021).

Sample extraction

The extraction process was performed in the laboratory of analytical research of the Department of Microbiology at Khwaja Yunus Ali University, Enayetpur, Sirajganj. A total sample of 100 g of the powdered leaves was weighed and placed into a Soxhlet extractor. The extraction was performed over 72 hours using 100 mL of 95% ethanol as the solvent, following previous protocols (Kaufmann et al., 2002; and Harborne, 1998). Additional extraction was carried out using a combination of 95% ethanol and water as solvents. The ethanol extract was concentrated by evaporating the solvent over a steam bath, and the final weight of the dried extract was recorded. The concentrated sample was labeled and stored in a sterile container at 4° C until further use.

Phytochemical analyses

Phytochemical analysis of the ethanol extract of tobacco was conducted to detect the presence of various bioactive compounds, including alkaloids, phenolic compounds, tannins, flavonoids, steroids, and terpenoids (Sofowora, 1993; Evans, 2009). Each compound was identified through standard phytochemical tests (Figure 1).

Carbohydrate test

The presence of carbohydrate was determined using Molisch’s test, which is considered a general test for carbohydrate (Parekh & Chanda, 2007). Briefly, two milliliters of an aqueous extract of the plant material were mixed with a 10% alcoholic solution of α-naphthol. Subsequently, two milliliters of concentrated sulfuric acid were carefully added along the side of the test tube to create a layer beneath the aqueous solution. A red or reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.

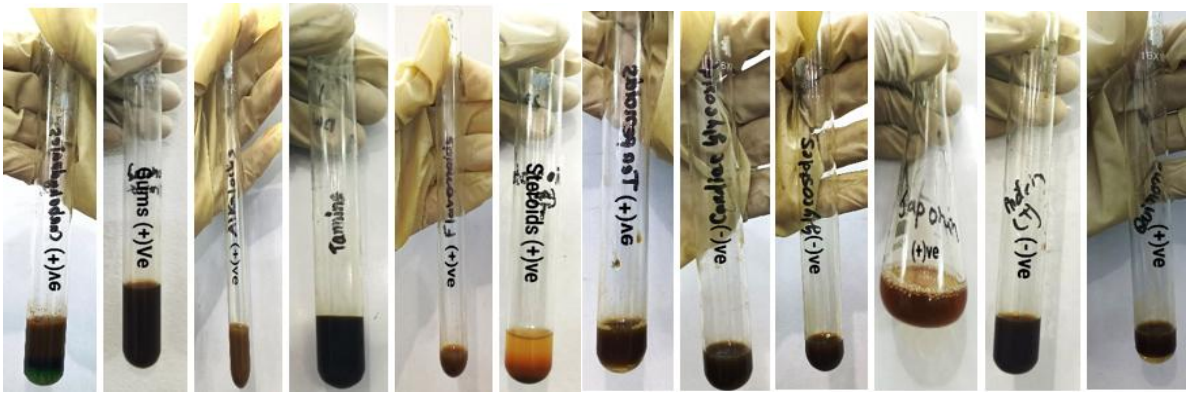


Figure 1: Phytochemical test of Tobacco leaves extract (Methanol)

Gums test

To detect the presence of gums, five milliliters of the extract solution was combined with Molisch reagent. Concentrated sulfuric acid was then added along the side of the test tube, forming a separate layer beneath the extract solution. The appearance of a red or reddish-violet ring at the interface of the two layers indicated the presence of gums (Dubale et al.,2023).

Alkaloid test

The presence of alkaloids was tested by separately adding Dragendorff's, Mayer's, and Wagner's reagents to three milliliters of filtrated extract in three test tubes. The formation of specific precipitates indicated the presence of alkaloids: an orange-red precipitate with Dragendorff's reagent, a creamy white precipitate with Mayer's reagent, and a reddish-brown precipitate with Wagner's reagent (Harborne, 1998).

Tannin test

To test for tannins, ten milliliters of filtrated extract were placed in a test tube, and 2–3 drops of a 0.1% ferric chloride ($FeCl_3$) solution were added. A brownish-green or blue-black color change indicated the presence of tannins (Sofowora, 1993).

Flavonoid test

To detect flavonoids, ten milliliters of filtrated extract were mixed in one milliliter of a 1% ammonium solution. The solution was shaken gently, and the appearance of a yellow coloration in the ammonia layer confirmed the presence of flavonoids (Edeoga et al., 2005).

Steroid test

To detect steroids, two milliliters of chloroform were added to 100 mg of extract, and the mixture was filtered into a test tube. Next, one milliliter of glacial acetic acid was added, followed by the careful addition of one milliliter of concentrated sulfuric acid (H_2SO_4) down the side of the test tube. The formation of a greenish color indicated the presence of steroid (Edeoga et al., 2005).

Terpenoid test

For the detection of terpenoid, five milliliters of chloroform were added to 100 mg of extract, and the mixture was filtered into a test tube. Then, three milliliters of sulfuric acid (H_2SO_4) were carefully added down the side of the test tube. The appearance of a reddish-brown color at the interface of two layers indicated the presence of terpenoid (Sofowora, 1993).

Cardiac glycoside test

To detect cardiac glycosides, one milliliter of extract was mixed with 0.5 mL of glacial acetic acid and 3 drops of a 1% aqueous ferric chloride solution. The formation of a brown ring at the interface of two layers indicated the presence of cardiac glycoside (Harborne, 1998).

Glycoside test

For the identification of glycoside, one gram of alcoholic extract from fresh or dried plant material was dissolved in one milliliter of water. A few drops of aqueous sodium hydroxide (NaOH) were added to the solution, and the appearance of a yellow color indicated the presence of glycoside (Evans, 2009).

Saponin test

To determine the saponin content, one milliliter of extract was added into nineteen milliliters of distilled water to make a final volume of twenty milliliters. The solution was mixed gently in a graduated cylinder for 15 minutes. The absence of foam formation indicated the absence of saponin in the extract in solution (Dubale et al., 2023).

Protein test

To identify the presence of protein, two milliliters of each extract were mixed with 0.5% sodium hydroxide solution and a few drops of 1% copper sulfate solution. The formation of a violet color indicated the presence of peptide linkages, confirming the presence of protein in the extract solution (Edeoga et al., 2005).

Quinone test

To check the presence of quinone in a solution, one milliliter of extract was mixed in one milliliter of sulfuric acid. The formation of a red color was considered an indication of the presence of quinone (Sofowora, 1993).

Antimicrobial sensitivity test of the leaf extract

In this current study, seven different microorganisms (gram-negative: Escherichia coli, Pseudomonas aeruginosa, and gram-positive: Streptococcus agalactiae, Serratia spp., Staphylococcus intermedius, and Staphylococcus warneri) were selected to evaluate the antimicrobial activity of Nicotiana tabacum leaf extract. Additionally, Candida species such as Candida albicans, Candida glabrata, and Candida krusei were also tested. The microorganisms were cultured on nutrient agar medium and sub-cultured on nutrient agar slants and incubated at 37° C for 24 hours.

Antimicrobial susceptibility test (Agar Well Diffusion Method)

The antimicrobial susceptibility of the test organisms was determined using the agar well diffusion method (Ansari et al., 2021). Muller-Hinton agar was prepared according to the manufacturer's instructions, autoclaved, and dispensed into sterile Petri dishes. The agar was allowed to solidify, and the plates were then inoculated with the test isolates. Using a sterile cork borer (5 mm diameter), three wells were made on each plate. The leaf extracts were added to each well at concentrations of 25 mg/µL, 50 mg/µL, and 75 mg/µL, corresponding to volumes of 50 µL, 100 µL, and 150 µL of extract, respectively. The inoculated plates were left at room temperature for 30 minutes to allow the extracts to diffuse into the agar. They were then incubated at 37°C for 18-24 hours. The antimicrobial activity was assessed by measuring the zones of inhibition around each well using a digital Vernier caliper.

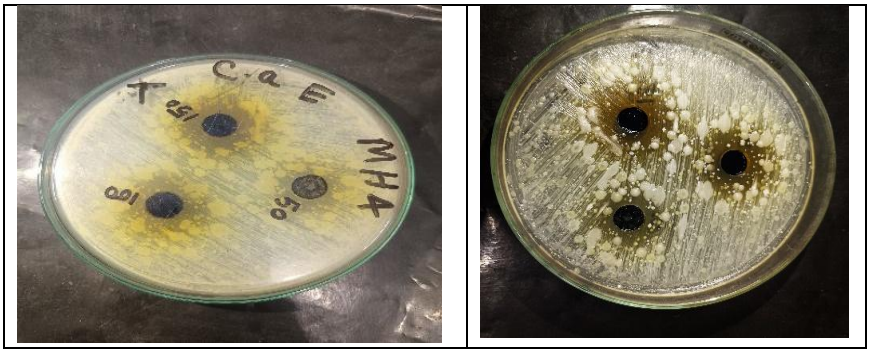
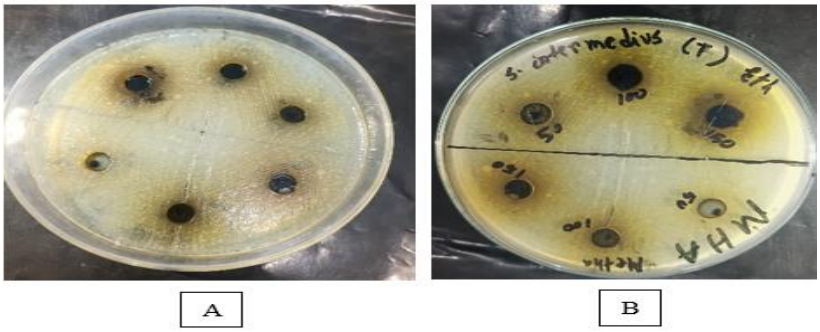


Figure 2: A and B are the *Candida albicans* which diameter are 20 mm, 25 mm and 30 mm at the concentration 50 µl, 100 µl, and 150 µl (Ethanol extract)



A B

Figure 3: The images of A and B for *Staphylococcus intermedius* did not show any zone any zone of inhibition in Tobacco extract in Ethanol and methanol.

Results and Discussion

The phytochemical screening of leaf extracts of *Nicotiana tabacum* revealed the presence of various bioactive compounds, which are summarized in Table 1. Different solvents (water, methanol, and ethanol) were used to extract these compounds, and their presence or absence was indicated by + (presence) or —. (absence).

Table 1: Phytochemical screening of the leaf extract of *Nicotiana tabacum.*

Name of the Test	Tobacco extract in water	Tobacco extract in methanol	Tobacco extract in ethanol
Carbohydrate	+	+	+
Tannins	+		-
Flavonoids	+	+	-
Saponins	+	+	+
Gums	-	-	+
Steroids	-	+	+
Glycosides	-	-	-
Alkaloids	+	+	+
Proteins	-	-	-
Carduiac Glycosides	-	-	+
Terpenoid	+	+	+
Quinone	+	+	+

The results of phytochemical analysis indicate that *Nicotiana tabacum* leaf extracts possess a variety of phytochemicals with potential therapeutic properties. All three extracts (water, methanol, and ethanol) exhibited the presence of carbohydrates, saponins, alkaloids, terpenoids, and quinones. Ethanol extract demonstrated that the highest number of positive results contained steroids, cardiac glycosides, and flavonoids in addition to the common phytochemicals shared by the other extracts.

Methanol extracts showed a similar composition, with the presence of saponins, flavonoids, alkaloids, terpenoids, and quinones, but no steroids or cardiac glycosides. The water extract, while less diverse, still contained carbohydrates, tannins, flavonoids, saponins, alkaloids, terpenoids, and quinones. These findings suggest that the different solvents used for extraction significantly impact the phytochemical profile, which can influence the potential medicinal properties of the plants. Further studies on the bioactivity of these compounds are needed to evaluate the therapeutic potentials of these extracts, particularly in the context of antimicrobial activity.

The phytochemical analysis of *N. tabacum* leaves (Table 1) revealed the presence of several bioactive compounds. Carbohydrates, which are known for their role as high-energy compounds, were found in significant concentrations across all extracts. Additionally, the leaf extract exhibited a high fiber content. A low moisture content is observed that suggests a longer shelf life for the plant materials, as it minimizes the risk of microbial activity during storage.

As seen in Table 1, alkaloids, flavonoids, quinones, saponins, and terpenoids were found in the *Nicotiana tabacum* leaf extracts, albeit in varying concentrations depending on the solvent used for extraction. Notably, the water and methanol extracts exhibited the highest concentrations of alkaloids and flavonoids, while quinones, saponins, and terpenoids were present in all three solvent extracts (water, methanol, and ethanol). These findings are consistent with previous studies (Kanmani *et al*., 2021).

The antimicrobial activity of *N. tabacum* leaf extract was evaluated against several microbial species using the agar well diffusion method. The results presented in Table 2 showed the zone of inhibition (diameter in mm) for the various concentrations (50 µl, 100 µl, and 150 µl) of the leaf extracts.

Table 2: Antimicrobial activity of *Nicotiana tabacum* leaf extracts

Organisms	50 µl Extract	100 µl Extract	150 µl Extract
Escherichia coli	35 mm	39 mm	47 mm
Staphylococcus intermedius	00	30 mm	35 mm
Candida albicans	15 mm	25 mm	35 mm
Candida glabarata	00	20 mm	28 mm
Candida krusi	00	00	25 mm

The antimicrobial activity of *Nicotiana tabacum* leaf extracts demonstrated the varying levels of inhibition against the tested microorganisms. The ethanol extract of *Nicotiana tabacum* exhibited significant antimicrobial activity that showed the highest zone of inhibition against *Escherichia coli* (47 mm at 150 µl concentration).

In contrast, the extracts did not show minimal or no effect on certain organisms, such as *Candida glabrata* at lower concentrations (50 µl) and *Candida krusei* at the concentrations of 50 µl and 100 µl. However, when the concentration increased to 150 µl, it showed a zone of inhibition, particularly against *Candida krusi* (25 mm). The results suggest that the extracts of *Nicotiana tabacum* leaf possess a strong antimicrobial property, which may be attributed to various bioactive compounds identified by phytochemical analysis, such as alkaloids, saponins, and

terpenoids. The higher concentrations of 100 µl and 150 µl appeared to enhance the antimicrobial effects, confirming the potential use of *Nicotiana tabacum* as a natural antimicrobial agent.

The antimicrobial activity of *N. tabacum* leaf extract in methanol was tested against various microorganisms. The results, presented in Table 3, show the zones of inhibition (in mm) at different extract concentrations (50 µl, 100 µl, and 150 µl).

Table 3: Antimicrobial screening of *Nicotiana tabacum* leaf extract in methanol on test organisms (mm)

Organisms	50 µl Extract	100 µl Extract	150 µl Extract
Streptococcus agalactiae	20 mm	25 mm	32 mm
Candida albicans	15 mm	25 mm	30 mm
Candida glabarata	R	20 mm	25 mm
Candida krusi	R	15 mm	35 mm

R = Resistant

As seen in Table 3, the antimicrobial activity of *Nicotiana tabacum* leaf extract in methanol exhibited significant inhibition against *Streptococcus agalactiae*, with zones of inhibition increasing from 20 mm at 50 µl to 25 mm at 100 µl and 32 mm at 150 µl. Similarly, *Candida albicans* increased inhibition with the extract concentrations, producing 15 mm at 50 µl, 25 mm at 100 µl, and 30 mm at 150 µl. Interestingly, *Candida glabrata* was resistant to the concentration of 50 µl but produced a zone of inhibition of 20 mm at 100 µl and 25 mm at 150 µl. *Candida krusi* also showed resistance at 50 µl but exhibited a significant zone of inhibition (35 mm) at 150 µl, indicating a potency for higher concentrations to be more effective against certain fungi. These results reinforce the effectiveness of *Nicotiana tabacum* leaf extract as an antimicrobial agent, particularly at higher concentrations. The differential response observed in the microorganisms tested further supports the need to optimize extract concentration and solvent choice for maximum antimicrobial efficacy. In table 3, we observed that *Streptococcus agalactiae* and *Candida albicans* were produced in zones in 50 µl, 100 µl, and 150 µl. *Streptococcus agalactiae* produced the highest zone, 25 mm in 100 µl and 32 mm in 150 µl. But *Candida glabrata* produced a zone 20 mm in 100 µl and 25 mm in 150 µl, and *Candida krusei* produced a zone of 15 mm in 100 µl and 35 mm in 150 µl.

The antimicrobial activity of *Nicotiana tabacum* leaf extract in ethanol was tested against several microorganisms, and the results are presented in Table 4. The zones of inhibition (diameter in mm) at different extract concentrations of 50 µl, 100 µl, and 150 µl (Table 4).

Table 4: Antimicrobial screening of *Nicotiana tabacum* leaf extract in ethanol.

Organisms	50 µl Extract	100 µl Extract	150 µl Extract
Escherichia coli	26 mm	28 mm	29 mm
Candida albicans	20 mm	25 mm	30 mm
Candida glabarata	25 mm	30 mm	35 mm
Candida krusi	15 mm	25 mm	30 mm

Table -4 highlights the antimicrobial activity of Nicotiana tabacum leaf extract in ethanol. Escherichia coli exhibited a consistent zone of inhibition across the concentrations, with the zones measuring in at 26 mm at 50 µl, 28 mm at 100 µl, and 29 mm at 150 µl. Similarly, Candida albicans showed inhibition by increasing concentrations, such as

a zone of 20 mm at 50 µl, 25 mm at 100 µl, and 30 mm at 150 µl. Candida krusei also exhibited inhibition of 15 mm at 50 µl, 25 mm at 100 µl, and 30 mm at 150 µl.

In this study, the phytochemical analysis of Nicotiana tabacum leaf extracts showed some similarities and differences when compared with previous research. For example, a study reported the presence of tannins in tobacco leaf extracts, while in our study, tannins were found to be absent in all three extract types (Lewis 2020).

However, other phytochemicals, such as alkaloids, flavonoids, saponins, and terpenoids, showed consistent results between both studies. The presence of these compounds suggests that the pharmacological potential of Nicotiana tabacum can be attributed to a wide range of bioactive phytochemicals, as seen in our findings.

Additionally, the antimicrobial activity observed in this study, particularly the zone of inhibition against Escherichia coli, shows similarities with the results of Dixit and Tiwari (2023). In both studies, Escherichia coli was found to be susceptible to tobacco leaf extract, confirming the antimicrobial properties of Nicotiana tabacum against common bacterial pathogens. Our study reported zones of inhibition of 35 mm, 39 mm, and 47 mm for Escherichia coli at extract concentrations of 50 µl, 100 µl, and 150 µl, respectively.

However, Candida glabrata showed the highest zone of inhibition, measuring 25 mm at 50 µl, 30 mm at 100 µl, and 35 mm at 150 µl. These results suggest that Candida glabrata is particularly sensitive to the ethanol extract of Nicotiana tabacum, with the highest antimicrobial effect recorded at the concentration of 150 µl.

These findings confirm that the antimicrobial screening of leaf extract of Nicotiana tabacum in ethanol exhibited the highest inhibitory effect on these microorganisms, especially Candida glabrata, and further validate the potential of tobacco as a potential source of antimicrobial agents.

Conclusion

The proximate analysis of *Nicotiana tabacum* leaves demonstrated that tobacco is a valuable source of carbohydrates and fiber, indicating its potential as a nutritional supplement, especially for cereal-based diets. The phytochemical analysis revealed the presence of a variety of bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids, which contribute to the medicinal properties of the plant. The antimicrobial screening further confirmed that *Nicotiana tabacum* leaves exhibit significant antibacterial and antifungal activities. These findings suggest that tobacco leaves possess considerable pharmacological and medicinal value, making them a promising candidate for the development of natural therapeutic agents against a wide range of bacterial and fungal infections.

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Authors' Contributions

The research concept and design were developed by Mohammad Zakerin Abedin and Md. Masudur Rahman Khalil. Materials and data collection were handled by Md. Shobuj Hossain and Md. Mursalin Hossain. Data analysis and interpretation were performed by Mohammad Zakerin Abedin and Md. Masudur Rahman Khalil. The literature search and article writing were conducted by Md. Masudur Rahman Khalil and Md. Shobuj Hossain. Critical review and article editing were done by Mohammad Zakerin Abedin and Abdullah Akhtar Ahmed. All authors approved the final version of the article.

Disclosure

The authors affirm that they have no conflicts of interest related to this study.

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