

Study of Antimicrobial Quality of Tobacco Leaves Extract (*Nicotiana tabacum*) on *Aeromonas hydrophila* and *Escherichia coli*

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<https://dx.doi.org/10.13005/bbra/3191>

(Received: 20 July 2023; accepted: 27 November 2023)

Tobacco is a perennial herb and its belong to family Solanaceae. Tobacco leaves have some antimicrobial substance such as alkaloids, phenol and some essential oils. The leaves extract of this plant show some microbial Quality and influence on both gram positive and gram negative bacteria. The aim of this work to determine the effectiveness of tobacco (*Nicotiana tabacum*) leaves extract in inhibiting on *Aeromonas hydrophila* and *Escherichia coli*. The antibacterial test performed on Mueller Hinton Agar (MHA) media. Test result indicate the leaves extract of tobacco showed positive result in *Aeromonas hydrophila*. Tobacco extract can inhibit *Aeromonas hydrophila* growth and a clear zone are found, in case of *E. coli* it showed negative result in both the cases. Tobacco extract cannot inhibit *E. coli* growth and the diameter of the inhibitory zone is not clear. The result shown that tobacco leaf extract can be source of an active antimicrobial substance.

Keywords: *Aeromonas hydrophila*; *E. coli*; Tobacco extract.

India is known for its Rich biodiversity region and have 91,000 animal's species and 45,000 plant species. As aqueous extract of medicinal plant has potential for antimicrobial activity for various microorganism¹. *Tobacco* is one of the perennial herbs and its belong to family Solanaceae. In total sixty species of *Nicotiana* few are homegrown to Australia, America in which *Nicotiana tabacum*, is use for commercial production. Exposure of *Tobacco* caused cancer and many disease in human but one of the good thing of *Tobacco* leaves have some antimicrobial substance such as alkaloids, phenol and some essential oils. The medicinal value of tobacco plant is known to 1492 to till now. *Tobacco* tea was used to kill intestinal worms, as well as for headaches, and used as an antiseptic.

Tobacco is known for cigarette smoking and it is continuing common practices in our society and it causes blood coagulation and increase cardiovascular disease². The leaves extract of this plant shows some antimicrobial Quality and influence on both gram positive and gram-negative bacteria.

Nicotine is the most important and active substance in *tobacco* product but clinical value is limited because it causes multiple negative effect. In many study it is found that nicotine has microbial viability on microbes and inhibitory effect on the growth of some microbes such as *Aeromonas hydrophila* and *E. coli*.

Aeromonas hydrophila is a gram-negative pathogen and usually found in fresh and brackish

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water. It cause many water born, intestinal and diarrheal disease in humans and also it infect animals such as fish ,Amphibians and mammals³. *Escherichia coli* is another gram-negative pathogen causes urinary tract infection and acute enteritis diarrhea. It is found in the food, environment and intestine of animals. At present our study show direct effect of Nicotine on microbes and it shows inhibitory effect. *Tobacco* extract can inhibit *Aeromonas hydrophilla* growth. In case of *E. coli*, it showed negative result in both the cases.

MATERIAL AND METHOD

Collection of samples

The Study conducted at the St. Aloysius College (Autonomous) Lab Jabalpur from January 2022 to May 2022. Tobacco leaf used for this research were obtained from local retailers at Adhartal market in Jabalpur state M.P. and identified at the Department of Zoology of St. Aloysius' College Jabalpur M.P., for removal of soil and dust particles the sample was wash with tap water and then again with distil water.

Preparation of extracts

Extract was prepared by weighing 50 grams of the homogenized leaf samples and 50 grams of the grounded snuff then it extracted with 500 ml of methanol using Soxhlet apparatus at 80!. After the extract was made it was collected in a sterile airtight glass bottle.

Preparation of media and plates

3.8 gm of Mueller Hinton Agar (MHA) powder diluted in 100ml of distilled water. Dissolve

all components by heating and continuous stirring. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes. The dissolved agar has been autoclaved, allowed to cool but not solidify. Pour MHA into each plate and leave plates on the sterile surface until the agar has solidified. Replace the lid of each Petri dish and store the plates in a refrigerator.

Cultures used

Aeromonas hydrophila and *E. coli* used as a test sample. For this purpose, culture obtained from Department of Zoology and Department of Microbiology of St. Aloysius' College respectively.

Inoculation

The inoculation was done by two methods streak plating technique and pour plating technique.

RESULT AND ANALYSIS

Results of the present study are presented in the following tables (Table1 & Table 2). We use *Aeromonas* culture plate to study the antimicrobial effect of tobacco extract.

Antimicrobial Effect of nicotine on *Aeromonas hydrophila*

The Result showed that tobacco leaves could inhibit the growth of *Aeromonas hydrophila* and *E. coli*. The study was conducted in replicates. Fresh cultures of respective pathogens were streaked over the surface of Mueller Hinton agar plates. 1 mg/ml extract was filled up of each well and well with solvent used as a negative effect. The plates were incubated at 37°C for 24 hours the results of the present study indicated antibacterial



Fig. 1. Preparation of Extract



Fig. 2. Preparation of Media

activity of tobacco leaves extract. The area of inhibition zone around the well were observe. Present study indicates the antimicrobial activity of tobacco leaves on *Aeromonas hydrophila*.

Antimicrobial Effect of nicotine on *Escherichia coli*

Extract of tobacco leaves does not have antimicrobial activity for *E. coli* and the diameter of the inhibitory zone is weak in comparative to *Aeromonas hydrophila*.

DISCUSSION

The antibacterial activity of tobacco leaves extracts against *Aeromonas hydrophila* (Gram positive) and *E. coli* (Gram negative) was measured by evaluating the diffusion method in agar.

Study performed by Anumudu CK et al2019 showed that the methanol extract of tobacco

leaves has great antimicrobial activity⁴. According to Sofi Mohammad Ashaq et al 2022 Organic solvents such as methanol, ethanol, acetone has bioactive compound⁵.The Present study exhibited



Fig. 3. Preparation of petriplates

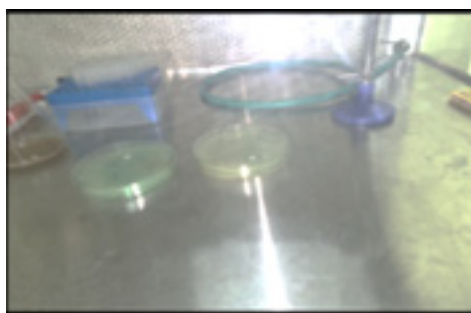


Fig. 4. Inoculation growth monitoring

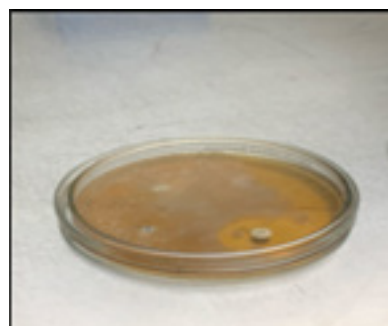


Fig. 5. Inhibition Zone

Table 1. Antimicrobial Effect of nicotine on *Aeromonas hydrophila*

No.	Name of Extract	Name	Description
1	Methanol	Media	Mueller Hinton Agar
2		Culture used	<i>Aeromonas hydrophila</i>
3		Extract used	2ml
4		Zone of Inhibition	Zone Visible
5		Antibiotic (Streptomycin)	Clear Zone Visible

Table 2. Anti-Microbial Assay of *Nicotiana tabacum* on *Escherichia coli*

No.	Extract	Name	Description
1	Methanol	Media	Mueller Hinton Agar
2		Culture used	<i>E. coli</i>
3		Extract used	2ml
4		Zone of Inhibition	Zone Not Visible
5.		Antibiotic (Streptomycin)	Clear Zone Visible

that, methanol extracts of the *Nicotiana tabacum* showed antimicrobial activity in different test organisms. Same result was found by K.P. Singh et al 2010⁶.

According to Nunes & Miguel 2017, Syadza Firdausiah et al, 2020 Tobacco leaves extract contain alkaloids, Phenol, terpenoid, flavonoids, Tannin compound, but did not contain Saponin⁷. According to A. Prommaban et al 2022 ethanolic extract of tobacco leaves has higher percentage of phenolic compounds than petroleum ether extracts⁸. The ability of these tobacco leaves extracts to inhibit the growth of *Aeromonas hydrophila* is due to presence of these secondary metabolites. Flavonoids Inhibit bacteria by produce of complex compound, Tanin react bacteria and inhibit the bacterial growth. Alkaloid compounds also inhabiting bacterial cell by disrupting the peptidoglycan.

In our study *Escherichia coli*. (gram negative) is a another test organism. In case of E.coli it showed negative result in both the cases. Tobacco extract cannot inhibit E.coli growth and the diameter of the inhibitory zone is not clear.

In recent study the result shown methanol leaves extract of tobacco are less effective in gram negative bacteria and it is very effective in gram positive bacteria⁹. The inhibition zone was clearer in gram positive bacteria may be due to the structure of cell wall is simpler as comparative to gram negative bacteria. The cell wall of gram-negative bacteria are more complicated so tobacco leaves extract not able to effect this cell wall and zone of inhibition is not cleared. This result is similar to the earlier study S A Fernanda et al 2021¹⁰. Earlier research have found that many phytochemicals present in medicinal plants are active agents for treatment of many disease caused by microbes and formulation of new drugs.¹¹

CONCLUSION

The present study shows that the methanol extract of tobacco leaves proves to be better in antimicrobial properties as compared to water extract. In recent study shows that methanol leaf extracts tobacco showed positive result in *Aeromonas hydrophila* as compare to E coli. Present study indicate that tobacco leaves extract can be used as a source of antimicrobial compound

for drug development and control of some bacterial agent.

ACKNOWLEDGEMENT

We thank Department Of Zoology and Department of Microbiology of St. Aloysius College Aloysius (Autonomous) for their support.

Conflict of interest

There is no conflict of interest for the article.

Funding Sources

There are no funding sources for this work.

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