Extraction and Purification of Nicotine Compound and Study of its Effect on Some Pathogenic Bacteria

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ABSTRACT:

Nicotine is a highly addictive alkaloid compound found in tobacco leaves. Nicotine has antibacterial properties, which make it a promising candidate for further research as a potential new antibacterial agent. This article provides an overview of the extraction and purification of nicotine from tobacco leaves using methods The antibacterial properties of nicotine are studied using different assays, which involve exposing bacterial cultures to different concentrations of nicotine and observing the effect on bacterial growth and viability. the results showed superiority of nicotine alkaloid over the aqueous extract that nicotine compound gave the highest inhibition against Pseudomonas in the highest concentration was the inhibition diameter 30 mm for Pseudomonas, 20 mm for coli. E and 20 mm for the Staph.

Keywords: isolation, diagnosis, purification, Nicotine, aqueous extracts, pathogenic bacterial.

استخلاص وتنقية مركب النيكوتين ودراسة تأثيره على بعض البكتيريا المرضية

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النيكوتين مركب قلويد شديد الإدمان موجود **الملخص:**

في أوراق التبغ ، النيكوتين له خصائص مضادة للبكتيريا ، مما يجعله مرشحًا واعدًا لمزيد من البحث كعامل جديد محتمل مضاد للجراثيم. تقدم هذه المقالة نظرة عامة على استخلاص وتنقية النيكوتين من أوراق التبغ باستخدام طرق تمت دراسة الخصائص المضادة للبكتيريا للنيكوتين باستخدام فحوصات مختلفة، والتي تتضمن تعريض الثقافات البكتيرية لتركيزات مختلفة من النيكوتين وملاحظة التأثير على نمو البكتيريا وحيويتها.

أظهرت النتائج تفوق قلويد النيكوتين على المستخلص المائي حيث أعطى مركب النيكوتين أعلى نسبة تثبيط ضد السيودوموناس في أعلى تركيز كان قطر التثبيط 30 مم للسيودوموناس و 20 مم للإكولاي. و 20 ملم للمكورات العنقودية.

الكلمات المفتاحية: عزل, تشخيص, تنقية, النيكوتين، المستخلصات المائية, الأجناس البكتيرية المرضية.

1. Introduction

Tobacco is a genera plants in the Solanaceae family, Nicotiana that originates in Australia, Southwest Africa, America, and the South Pacific region (Lewis, 1931). Nicotine is an alkaloid compound found in the nightshade family of plants, including tobacco. It is a highly addictive substance and has been linked to several health

problems, including respiratory and cardiovascular diseases as well as cancer. It has a significant economic value and is grown in over 120 countries (Wang and Bennetzen, 2015). Despite the fact that over 75 tobacco species have been identified, only Nicotiana rustica L. and Nicotiana tabacum L. are currently commercially farmed for human use (Tezuka et al., 2010). Several other species are also cultivated for decorative or industrial uses (Abdullah et al., 2020). N. tabacum is one of the most famous plant kingdom species with chemical and biological significance, having over 2500 characterized metabolites currently in existence, and is also widely farmed globally (.Jassbi et al.,2017).

Each year, around 6 trillion cigarettes are used worldwide, and according to a 2019 report, total tobacco output was approximately 6.68 million metric tons, with China being the largest tobacco producer worldwide that year (Shahbandeh, 2023). As a result, such massive production generated trillions of tobacco manufacturing chemical wastes, including nicotine, each year, negatively impacting the environment. Although it is required that the tobacco industry maintain a landfill, specifically for the disposal of tobacco waste, environmental rules are rarely followed in many wealthy countries (Mumba,2008). However, stories of tobacco phytochemicals being used for other purposes bring up various study avenues.

Although recycled tobacco fiber can be used to make restored tobacco newspaper, it would result in the waste of several phytochemicals with valuable functions such as anti-inflammatory capacity, antioxidant properties, and anti-fungal activity (Bano et al.,2020).

Nicotine, also known as 3-(1-methyl-2-pyrrolidinyl) pyridine (Fig. 1), is a prominent and common alkaloid found in tobacco extracts. Nicotine is created in tobacco plants by the ornithine metabolism (Civilini et al.,1997), and it is stored in the leaves. Another proof is that there is no nicotine.

The mechanism of action for nicotine's antibacterial properties is not fully understood, but it is thought to involve disruption of bacterial cell membranes and inhibition of bacterial growth.

Studies have shown that nicotine is effective against both planktonic (free-floating) and biofilm forms of bacteria, which is important as biofilms can be more resistant to antibiotics.

Some studies have also shown that nicotine can enhance the activity of antibiotics against certain bacteria, suggesting that it could potentially be used in combination therapy with antibiotics. According to Akinpelu and Obuotor, 25 mg/mL of N. tabacum leaf extract inhibits the growth of both gramme positive and gramme negative bacteria, including *Bacillus subtilis, Corynebacterium pyogenes*, *Pseudomonas aeruginosa, Serratia marcescens, Shigella dysenteriae, and Staphylococcus aureus* to be equally effective as an antibacterial agent as the positive control, gentamicin.

Nicotiana rustics' leaves are used to treat bronchitis, tonsillitis, wounds, sore throats, and arthritis. Meanwhile, its extract has been shown to be beneficial in treating respiratory tract illnesses (Bussmann et al.,2020).

Furthermore, Nicotiana tabacum leaves have comparable activities and are still beneficial in treating digestive system diseases, skin illnesses, sinusitis, and stomach infections (Delbanco and Burgess obacco,2017) extract can halt nosebleeds and cure bilharzia in Madagascar (Razafindraibe *et al*.2013).

2. MATERIALS AND METHODS:

2.1. Nicotine (NCT) Extract Preparation

2.2.1. Tobacco Leaf Extraction the top, middle, and bottom regions of N. tabaccum leaves were dried for 2 hours in a 55°C oven, crushed to a powder. The dry N. tabaccum leaves were extracted using water maceration. The extract from the maceration was combined, filtered, and evaporated using a rotary evaporator.

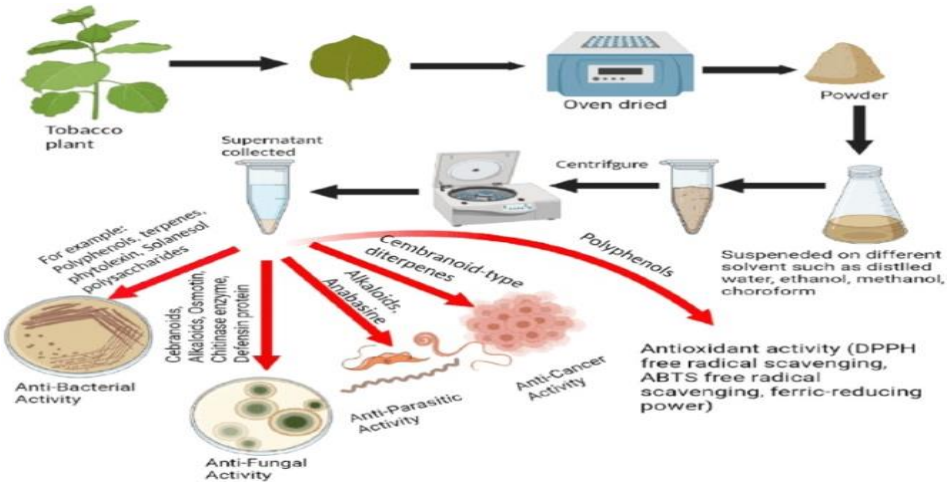

Fig.1.straightforward diagram of tobacco leaf extraction and biological application

The acid-base extraction method is based on nicotine's alkaloid characteristic, which entails different amounts of solubility in water and an organic solvent. The amount of 50 g of tobacco leaves was boiled with 750 mL of water at 80 ± 5 ◦C for 20 min, then 10 g of sodium carbonate was added and the mixture was continuously heated for 10 min Following filtration, the acquired Sodium hydroxide was used to adjust the filtrate to pH 12 before extracting it *with* chloroform (100 mL twice) using the liquid-liquid extraction procedure. To get crude extracts, the filtrate was extracted using a rotary evaporator at 50 °C under vacuum (Tantullavetch et al.,2007). Each extraction process was repeated twice. The crude extracts were kept at 4 °C and kept away from light for further investigation. The yield of tobacco leaf extract is calculated using Equation (1).

$$\text{Yield of tobacco leaves extract} = \frac{\text{Weight of extract}}{\text{Dry weight of tobacco leaves}} \times 100\% \quad (1)$$

2.1.1. Calculation of NCT Content in Extracts

The NCT yield % was determined using an Agilent HP1100 HPLC instrument with an auto sampler. The stationary phase was a C-18 reverse-phase column with a diameter of 4.6 150 mm. The mobile phase was sodium acetate solution, (88v/v), with the pH adjusted to 4.2 using glacial acetic acid, at a flow rate of 1 mL/min, and UV detection

at 259 nm (Boateng and Okeke, 2019). The retention time of NCT was around 3.2 minutes. From NCT standards ranging from 5 g/mL to 250 g/mL in water (R2 = 0.9999) and ethanol (R2 = 0.9997), a calibration curve was produced. Equation (2) depicts the computation of NCT per tobacco leaf extract yield:

$$\text{Yield of NCT content in the extract} = \frac{\text{Weight of NCT}}{\text{Weight of tobacco leaves extract}} \times 100\% \quad (2)$$

2.1.2. NCT Fast Dissolving Films: Creation and Characterization

Preparation of NCT Fast Dissolving Film

The solvent casting technique was used to create the NCT quick dissolving films. HPMC E15 was dissolved in distilled water, stirred for 30 minutes at room temperature (25 °C), and then diluted to a hydro alcoholic solution (ethanol: water = 9:1) containing 5% by weight of HPMC E15. After gently stirring the HPMC E15 solution for 5 minutes, 0.51% w/w NCT extract was added and allowed to sit until all visible air bubbles had vanished. Ten grams of the produced solution were placed on a 63.64 cm2 Petri dish with a 9 cm diameter and allowed to dry at room temperature overnight.

2.2. Bacteriological sources

PBSA (Phosphate-buffered Saline Agar) in 1 liter of phosphate-buffered saline (PBS) (Oxoid), l0g of proteose peptone (Oxoid) and 10g of agar (Oxoid) were dissolved. Following heat sterilization, 3 mg nicotinamide adenine dinucleotide (NAD) (British Drug Houses-BDH) and 3 mg haemin (BDH) were added, both filter sterilized. The resultant medium only moderately promoted the growth of Staphylococcus, Pseudomonas and E. coli.

2.3. Stimulation tests for tobacco

Tobacco was placed in the bottom of a petri dish and coated with 20 ml of PBSA. After the plates had been established, they were inundated with an overnight broth culture of the organism diluted 1/100 in PBS. The excess was drained, and the plates were dried and incubated at 37° for 18 hours. PBSA plates were also prepared, but without the tobacco in the medium, and seeded with Staphylococcus, Pseudomonas and E. coli. When the plates were dry, a little piece of tobacco was placed on top of the agar and the plate was incubated at 37° for 18 hours. Tobacco aqueous extract was produced in distilled water at a concentration of 5 mg per ml and left at room temperature for 1 hour. PBSA plates were poured, and the surfaces were seeded with organisms as before. The agar was then sliced into wells and filled with aqueous tobacco extract.

2.3.1 Stimulation tests for nicotine

Pure nicotine dilutions ranging from 0-1% to 0-03% were made in 10-ml aliquots of molten PBSA and put into petri dishes. After they had hardened, a 10 ml layer of plain

PBSA was placed on top. The Staphylococcus, Pseudomonas and E. coli broth culture was distributed in a consistent manner. For 18 hours, the incubator was set to 370°F. Plates were loaded with 20 ml of PBSA and surface seeded with diluted Staphylococcus, Pseudomonas and E. coli broth cultures, and a well was cut and filled with a 1% solution of pure nicotine. Both the submerged tobacco and nicotine (in well) stimulation assays were replicated in a petri dish using bacterial agar.

2.4. Statistical Analysis

ANOVA was used to evaluate the percentage extract yield and NCT content in tobacco leaves, and the unpaired t-test was used to characterize the NCT rapid dissolving films using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). The data are provided as mean SD, with p 0.05 indicating significant differences.

3. Results and discussion

3.1. NCT Fast Dissolving Films

The results of the NCT rapid dissolving films' thickness, weight, and disintegration time are displayed in Table 1. There was a distinction between the Disintegration time and film weight between NCT films and blank films (p 0.05).

The NCT film disintegrated more slowly than the blank films as the film weight was increased. The findings suggested that NCT might have an impact on the structure of polymeric films. Choi and others (2016)

Table 1. Yield extract percentage and nicotine (NCT) content from water maceration extraction method and parts of tobacco leaves.

Items*	
Yield of Tobacco Leaf Extract %	45.78 ± 1.60
Yield of NCT Content in the Extract%	6.35 ± 0.80

*** Statistically not different (p > 0.05).**

Table 2 displays mechanical characteristics and NCT loading effectiveness. This outcome demonstrated that the inclusion of NCT and tobacco leaf extract in the film had significantly higher values for the Young's modulus, % elongation at break, and tensile strength. This outcome contrasted with the oily, drug-like essential oil that is typically included in formulations and affects the mechanical characteristics of the film by reducing tensile strength and increasing brittleness (Shojaee et al., 2014).

The NCT fast-dissolving films disintegrated in less than 30 s, which is a respectable amount of time for a fast-dissolving film.

Table 2. Thickness, weight, and disintegration time of NCT fast dissolving films.

Film	**NCT Film ***	**Blank***
Thickness (mm)	0.070 ± 0.001 [a]	0.078 ± 0.001 [a]
Weight (g)	0.0313 ± 0.001 [a]	0.0255 ± 0.001 [b]
Disintegration Time (s)	18.98 ± 0.90 [a]	11.50 ± 1.05 [b]
Normalized Disintegration Time (s)	18.96 ± 1.00 [a]	10. 70 ± 1.10 [b]

***"a" and "b", at the same raw are statistically different (p < 0.05).**

Table 3. Mechanical properties and NCT loading efficiency of NCT fast dissolving films.

Film	**NCT Film ***	**Blank***
Tensile Strength (N/mm2)	7.55 ± 0.25 [a]	4.10 ± 0.05 [b]
Elongation at Break (%)	7.60 ± 0.04 [a]	4.80 ± 0.20 [b]
Young's Modulus (N/mm2)	220.90 ± 6.30 [a]	120.60 ± 4.60 [b]
NCT Loading Efficiency (%)	96.01 ± 5.10	-

***"a" and "b", at the same raw are statistically different (p < 0.05).**

Table 4. Results of biochemical tests for pathogenic bacterial isolates.

	Isolated Bacteria		
	Staphylococcus	Pseudomonas	E. coli
Gram strain	+	–	–
Catalase	+	+	+
Oxidase	–	+	–
I	–	–	+
M	–	–	+
V	–	–	–
C	+	+	–
Urease	+	+	–
Gelatin	+	+	–

Nitrate reduction	+	+	+

3.2. Diagnosis of bacteria: These isolates were identified by several from the tests and using culture media to note the shape Growth and discriminatory traits were classified into group's Taxonomic classification according to Bergey's manual and refers to (Holt *et al*., 1994) Table 4

3.3. Effect of aqueous extract of tobacco leaves

The compound showed significant differences and good biological activity Against bacterial isolates, as it was noted that the compound has an inhibitory effect And for all concentrations, and through the biological study, it was noted that The concentration is 1 mg/ml, which is an inhibitor for all isolates Positive or negative for gram stain, In comparison with the effect Inhibitory of the aqueous extract of tobacco leaves note that the concentration The lowest level is not inhibitory for all bacterial isolates. Results it was noted that the compound gave the highest inhibition against Pseudomonas in the highest concentration was the inhibition diameter 30 mm for Pseudomonas, 15 mm for coli. E and 15 mm for the Staph. Aurous. In Table 2 and Fig.2.

The aqueous extract of tobacco leaves was obtained It was prepared at a concentration of 100 mg / ml, and from it the rest of the concentrations were prepared Which was represented by 10, 20 , 30, 40 and 50 mg/ml in general, the effectiveness against bacterial of the extract depended on the type of microorganism, and it has been proven by the current study, the aqueous extract showed good efficacy The ability of the aqueous extract to have a biological effect is due to the presence of alkaloid compounds, which have antimicrobial activity

Hence the importance of the compound in that it inhibits the growth of Gram-positive and gram-negative bacteria, its effectiveness returns it is an alkaloid compound and the alkaloids are known for their toxicity (Mansk, 1950) High or it may be due to the inability of the bacterial membrane to block the entry of the extract into the bacteria and inhibiting its activity, including the extract contains inhibitory substances.

Further studies reported that tobacco leaf extract was found similarly, two separate studies in Pakistan found that N. tabacum extracts have substantial antibacterial action against gramme positive S. aureus (Bakht et al.,2012). Sharma and colleagues demonstrated that an acetone stem extract of tobacco inhibits the growth of E. coli, S. aureus, P. aeruginosa, and B. amyloliquefaciens, while methanol, aqueous, and ethanol extracts of tobacco tissue were also effective against E. coli (Sharma et al.,2016).

An Ethiopian investigation found that 100 and 200 mg/mL plant extracts of N. tabacum have an antagonistic effect against S. aureus, S. agalactiae, S. dysgalactiae, and Dermatophilus congolensis [Kalayou et al.,2012).

A group of researchers recently demonstrated that crude extracts of N. tabacum have antibacterial efficacy against biofilm forming uropathogens, with clinical isolates being the most resistant (Ameya et al., 2017).

Similarly, a recent Saudi Arabian study reported that N. glauca leaves and flower extracts inhibited the growth of E. coli and S. aureus (Ali, 2021), implying that N. glauca leaves and flower extracts could be used for the treatment of bacterial infections, and the plant could be used for medicinal and economic purposes.

Table 5. Aqueous extract of the tobacco plant and its effects on bacterial growth

Concentrate level Mg/ml	Isolated Bacteria		
	Staphylococcus	Pseudomonas	E. coli
10	1 ± 0. 0 1	8± 0.50	2± 0.10 *
20	4± 0.20	15± 0.80	6± 0.30
30	8± 0.50	24± 1.10	10± 0.80
40	15± 0.90	30± 2.00	15± 1.20
50	25± 1.20	36± 4.00	20± 1.70

*** (p > 0.05). Average ± SE (standard error**

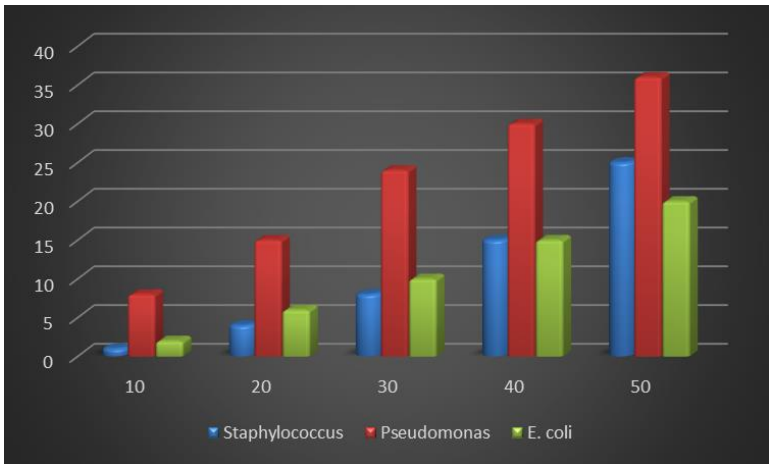

Fig.2 Aqueous extract of the tobacco plant and its effects on bacterial growth.

Table6. Effect of the nicotine compound and its effects on bacterial growth

Concentrate level Mg/ml	Nicotine compound		
	Staphylococcus	Pseudomonas	E. coli
10	2± 0.01	4± 0. 10	2± 0.01
20	5± 0.60	11± 0.80	6± 0.80

30	10± 0.80	21± 1.10	10± 0.80
40	14± 1.00	25± 2.00	20± 1.20
50	20± 1.50	30± 2.40	30± 2.00

* ($p > 0.05$). Average ± SE (standard error).

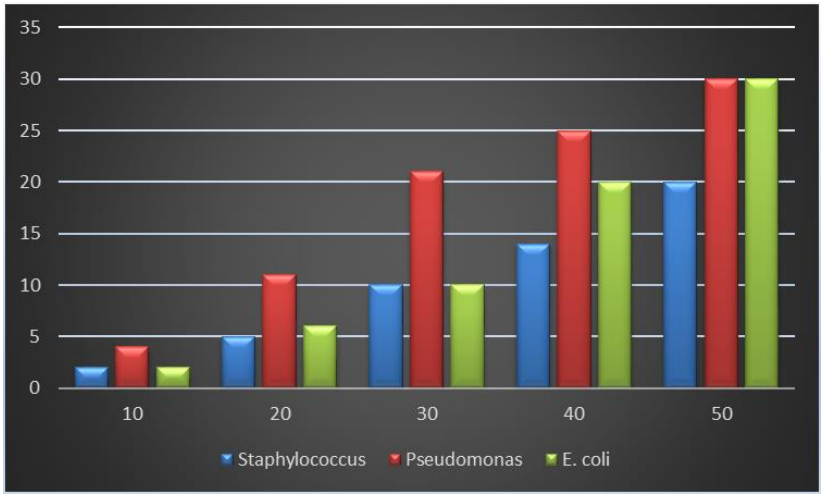

Fig.3 the nicotine compound and its effects on bacterial growth.

3.4. Effect of the nicotine compound

Results it was noted that the nicotine compound gave the highest inhibition against Pseudomonas in the highest concentration was the inhibition diameter 30 mm for Pseudomonas, 20 mm for coli. E and 20 mm for the Staph. Aurous. (Table 3, Fig.3 and Fig.4).

Many results were found that tobacco as well as quinic acid and caffeic acid esters. Most of chromogenic acid and aglycone reach the colon together, and release a large number of bioactive substances such as protocatechuic acid (PA), quinic acid, dehydroquinic acid (dhqa), dihydroferuloylquinic acid (dhfqa), and dihydroxycyclohexane (Pinta et al.,2018). These catabolic reactions necessitate the participation of numerous microorganisms, some of which must be reinforced Polyphenols' prebiotic activity has been extensively recognized as a health-related concern, with some dietary polyphenols not only enhancing the growth of probiotics such as Lactobacillus and Bifidobacterium but also inhibiting the reproduction of harmful bacteria.

Many chemicals found in tobacco plants, including polyphenols, terpenes, phytolexin, and polysaccharides, among others, have antibacterial properties. Many of these actions are naturally secreted by Nicotiana plants. Several research have confirmed that Nicotiana plants can be employed as a powerful antibacterial agent (Malik et al.,2015).

In this context, polyphenols are decomposed by the intestinal microbiota into metabolites, and polyphenols also regulate the balance of the intestinal microbiota and suppress the production of harmful microbial toxins (Dey,2019).

Some polyphenols, including quercetin, are conjugated with rutinose, and the related metabolites are glycoside quercetin and rutin. In general, the sugar portion cannot be digested by intestinal -glycosidase, hence the metabolism of intestinal microbiota such as Bacteroides, Blautia, and others is required.

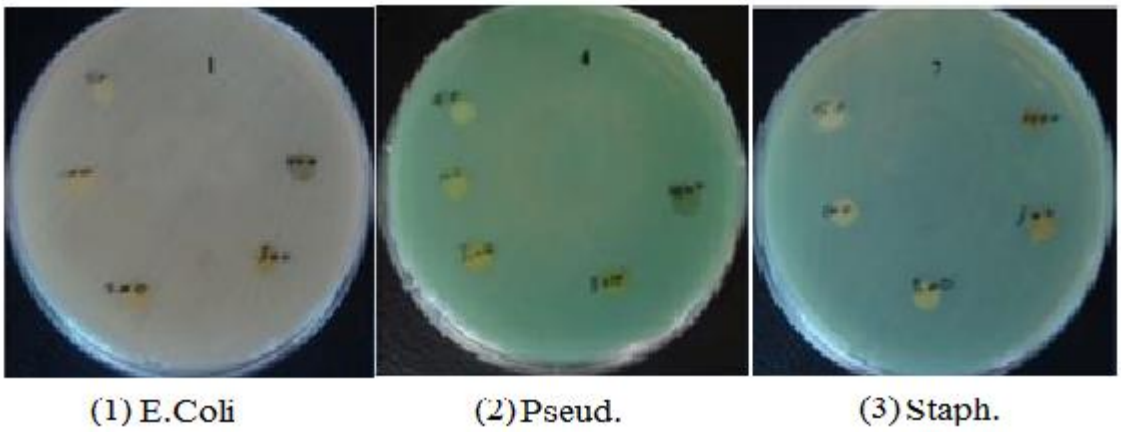

Fig.4 Inhibitory effect of nicotinic compound of tobacco against bacteria

4. CONCLUSION:

The by-products of the tobacco manufacturing business have a high potential value due to their wide range of application scenarios. To minimize harmful and side effects, the first concern in the development of new tobacco-related goods is the harm that nor nicotine can do to the human body. Second, the impact of intestinal flora on tobacco bioactive compounds should be examined for health products derived from tobacco waste, with tobacco fermentation products being a potential study direction. Finally, in the industry, the effect of the extraction procedure on the outcomes is crucial. Each extraction process must have its own selectivity and limitations.

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