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Antibacterial Activity, Chemical Profiling, and Safety Assessment of Nicotiana tabacum L. Extract against Pseudomonas aeruginosa in Otitis Media

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ABSTRACT

Background: Chronic suppurative otitis media (CSOM) remains an important cause of long-standing ear disease and hearing impairment, with *Pseudomonas aeruginosa* frequently implicated in persistent infection. This study investigated the chemical constituents, elemental profile, and antibacterial activity of an ethanol extract prepared from *Nicotiana tabacum* L. leaves against clinical organisms associated with ear infection.

Methods: Authenticated *N. tabacum* leaves were subjected to cold maceration in ethanol. The resulting extract underwent preliminary phytochemical screening, chromatographic fractionation, GC–MS and LC–MS profiling, and atomic absorption spectrometric analysis for selected elements. Antimicrobial activity was examined by agar-well diffusion against *P. aeruginosa*, *Escherichia coli*, Klebsiella spp., *Staphylococcus aureus*, *Proteus mirabilis*, and *Candida albicans*.

Results: The extraction process gave a yield of 2.24% w/w. Screening revealed alkaloids, flavonoids, cyanogenic glycosides, carbohydrates, phenolic constituents, and steroid/triterpenoid compounds. The crude extract inhibited *P. aeruginosa* in a concentration-related manner, producing zones ranging from 20.00 ± 2.646 mm at 100 µg/mL to 9.67 ± 1.528 mm at 6.25 µg/mL, while the remaining test organisms showed no measurable inhibition. Activity declined markedly after fractionation, with a maximum zone of 4.00 ± 0.44 mm. Atomic absorption analysis detected lead (0.015 mg/L), arsenic (<0.001 mg/L), manganese (0.06 mg/L), and cadmium (0.0023 mg/L); mercury, cobalt, nickel, and copper were not detected. GC–MS produced putative annotations for 16 constituents, mainly esters and fatty-acid-related compounds, whereas LC–MS tentatively assigned 10 metabolites, including nicotine, nornicotine, anabasine, chlorogenic acid, kaempferol, and quercetin.

Conclusion: The ethanol leaf extract of *N. tabacum* demonstrated selective in-vitro activity against the clinical *P. aeruginosa* isolate. The weaker activity of the separated fractions compared with the crude extract suggests that interactions among several constituents may contribute to the antibacterial effect.

1. Introduction

Chronic suppurative otitis media (CSOM) is a long-lasting inflammatory condition of the middle ear that is commonly associated with a perforated tympanic membrane and recurrent or continuous discharge from the ear. Although it may receive less clinical attention than acute otitis media, its effects can be considerable, especially among children and communities where access to specialist ear services is limited. Available epidemiological evidence indicates that CSOM remains a significant global health problem, with the greatest burden occurring in low- and middle-income countries.[1] A recent systematic review estimated a pooled worldwide prevalence of about 3.8%, representing roughly 297 million people, and noted that a substantial proportion of affected individuals experience disabling hearing loss.[2] The consequences of CSOM are not limited to persistent otorrhoea. Ongoing inflammation, perforation of the tympanic membrane and damage to the ossicles may progressively impair hearing, while severe or neglected disease can extend beyond the middle ear and produce more serious complications.[3] The microorganisms recovered from CSOM vary with geographical setting, patient characteristics and previous antimicrobial exposure, but *Pseudomonas aeruginosa* and *Staphylococcus aureus* are

repeatedly reported among the important bacterial pathogens[4] Biofilm formation is also increasingly recognized as a factor that allows organisms such as *P. aeruginosa* to persist and become less responsive to treatment.[5,6] Management generally involves cleaning of the ear together with topical antimicrobial treatment, while surgical intervention may be required in selected persistent or complicated cases. Although topical fluoroquinolones remain useful therapeutic options, resistance, biofilm-associated persistence, adherence difficulties and limited specialist access can contribute to treatment failure.[7] These challenges justify continued exploration of alternative sources of antibacterial compounds.

Plants continue to attract interest in antimicrobial research because they contain chemically diverse secondary metabolites, including alkaloids, phenolic acids, flavonoids, coumarins, terpenoids and fatty-acid derivatives. Such compounds may interfere with several bacterial processes, including membrane integrity, oxidative balance, quorum sensing, enzyme activity, virulence and biofilm development. Their natural origin, however, does not automatically make them safe; chemical characterization and appropriate toxicological assessment remain necessary. *Nicotiana tabacum* L., a member of the Solanaceae family, illustrates this dual nature. Tobacco leaves contain potentially useful phytochemicals alongside biologically active alkaloids with recognized toxicological significance. Reported constituents include nicotine and nornicotine as well as phenolics, flavonoids, terpenoids and other specialized metabolites.[8,9] The chemical composition can vary considerably with cultivar, plant age, tissue type, growing conditions, solvent selection and processing methods.[10,11] Previous investigations have shown that *N. tabacum* extracts can exhibit antibacterial activity and that the degree of activity may depend on the extraction solvent.[12,13] The present work therefore combined phytochemical screening, antimicrobial testing, chromatographic separation, GC–MS, LC–MS and elemental analysis to examine an ethanol leaf extract. The purpose was to establish whether the extract showed reproducible *in-vitro* activity against clinically isolated organisms associated with ear infection and to generate chemical leads for future bioassay-guided studies, rather than to claim clinical efficacy in CSOM.

2. Materials and Methods

2.1 Study design

This was an experimental laboratory investigation organized into five related stages: preparation of an ethanol extract from *N. tabacum* leaves; qualitative phytochemical screening; in-vitro antimicrobial testing using clinical ear-infection isolates; chromatographic separation with subsequent GC–MS and LC–MS profiling; and elemental analysis of selected potentially toxic metals by atomic absorption spectrometry.

2.2 Plant material and authentication

Leaves of *Nicotiana tabacum* L. were obtained from Oja-Oba Market, Akure, Ondo State, southwestern Nigeria, in September 2023. The reported collection coordinates were approximately 7°15′0″ N and 5°11′42″ E. Botanical authentication was carried out by a taxonomist in the Pharmacognosy Department Herbarium, Faculty of Pharmaceutical Sciences, University of Port Harcourt, and voucher number UPH50627 was assigned. The collected leaves were washed with distilled water, dried under shade at room temperature, for 2 weeks and pulverized using a machine fitted with a reported 200-µm sieve.

2.3 Extraction

Two hundred grams of the powdered leaves were soaked in 2 L of 70% ethanol for 72 h at room temperature, with intermittent agitation. The reported room temperature was approximately 27–29°C. The resulting macerate was passed through two layers of muslin cloth, centrifuged at 9,000 rpm for 10 min, and filtered again through Whatman No. 4 paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 50°C and 100 mbar. After drying, the extract was weighed and the percentage yield calculated. The dried material was kept refrigerated at approximately 2–8°C until analysis.

2.4 Preliminary Phytochemical Screening

The crude extract was screened qualitatively for major classes of secondary metabolites using established colour and precipitation reactions[14] Alkaloids were investigated with Dragendorff's, Mayer's and Hager's reagents. Flavonoids were assessed using Shinoda and aluminium-chloride reactions, while tests were also performed for anthraquinones, saponins, cyanogenic glycosides, carbohydrates, phenolics, phlobatannins, cardiac glycosides, triterpenoids/steroids and fixed oils. Fehling's and Molisch reactions were used for carbohydrates; Keller–Killiani and Kedde reactions for cardiac glycosides; and Liebermann–Burchard and Salkowski reactions for triterpenoid/steroid constituents.

2.5 Test Microorganisms

The microorganisms examined were *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, Klebsiella spp., and *Candida albicans*. These

organisms had been clinically isolated from patients with ear infections at the ENT Department of the University of Port Harcourt Teaching Hospital, Nigeria. Bacterial cultures were maintained on nutrient agar, while the yeast was maintained on malt-yeast agar before testing.

Isolation of test organism

The ear swab was collected using sterile swab and immediately transported in Stuart medium at 2 – 8^{0}C. For gram negative organisms, the specimen was streaked onto MacConkey Agar and incubated at 35 – 37^{0}C for 18 – 24 hours. For gram positive organism the specimen was streaked Mannitol Salt Agar, incubated at 35 – 37^{0}C for 18 – 24 hours, *Candida* specie: the specimen was streaked onto Sabouraud Dextrose Agar supplemented with chloramphenicol incubated at 30 – 37^{0}C for 24 - 48 hours Only the first isolate of each patient was used within 2 days. Gran staining was used to identify the organisms. *Candida* organisms were identified by direct microscopic examination, by preparation of a wet mount.

2.6 Antibacterial assay

Antibacterial activity was determined by the agar-well diffusion as an initial screening method. Fresh 24-h bacterial cultures were suspended in sterile 0.85% saline and adjusted to approximately 10^6 CFU/mL using a 0.5 McFarland standard. About 100 µL of the standardized suspension was repeatedly (3x) spread over the agar surface to produce confluent growth. The dried extract was reconstituted in 20% dimethyl sulfoxide (DMSO to produce 100, 50, 25, 12.5 and 6.25 µg/mL) Ciprofloxacin ear drops served as the positive control, and 20% DMSO was used as the negative control. Following application of the test preparations, the agar plates were left briefly to allow diffusion and were then incubated aerobically at 37°C for about 24 h. Inhibition zones were measured in millimetres, with each determination carried out in triplicate.[21]

Concentration-Response Antibacterial Assay

To examine the relationship between concentration and antibacterial response, the crude ethanol extract was dissolved in 20% DMSO and tested at 100, 50, 25, 12.5 and 6.25 µg/mL.

2.7 Antifungal assay

Candida albicans was additionally assessed using a microplate dilution procedure, with fluconazole serving as the reference antifungal drug.[22,23] The plates were incubated for 3–5 days at approximately 35°C. Since the crude extract produced no measurable inhibition zone against *C. albicans* in the principal agar diffusion experiment, these antifungal observations were treated as supplementary rather than as the main outcome of the study.

2.8 Bioassay-guided chromatographic fractionation

Bioassay-guided separation of the ethanol extract was performed by silica-gel column chromatography. About 2.46 g of extract was adsorbed onto 50 g of silica gel (60–120 mesh) and loaded onto the column. Five fractions, designated CF1–CF5, were collected and examined for antimicrobial activity. Thin-layer chromatography was used to compare the fractions under ultraviolet light at 254 and 365 nm and after iodine visualization. Fractions with similar chromatographic behaviour were combined when appropriate. CF4 was selected for further purification and spectroscopic investigation.

The antimicrobial results obtained after fractionation showed that the separated fractions were considerably less active against *P. aeruginosa* than the original crude extract. Because *P. aeruginosa* was the microorganisms mostly implicated in chronic *otitis media*. This pattern raises the possibility that the antibacterial effect may depend on additive or synergistic interactions among constituents, rather than on the activity of one isolated compound alone.

2.9 Heavy-metal/elemental analysis

For elemental analysis, approximately 50 mg of the sample was treated with a 1:1 mixture of hexane and dichloromethane and subjected to silica-based clean-up. The preparation was subsequently treated with nitric acid and refluxed at approximately 95 ± 5°C before dilution and filtration. The final solution was analysed by atomic absorption spectrometry for lead, arsenic, manganese, cadmium, mercury, cobalt, nickel and copper.

2.10 GC–MS analysis

GC–MS profiling was carried out with an Agilent gas chromatograph coupled to a mass spectrometer and fitted with a DB-5MS fused-silica capillary column measuring 30 m × 0.25 mm with a 0.15 µm film thickness. Using standard procedure: A 1-µL aliquot was introduced in pulsed splitless mode, using helium as the carrier gas at approximately 1 mL/min. The oven was held at 55°C for 0.4 min and subsequently programmed to 200°C at 25°C/min. Putative compound assignments were based mainly on retention characteristics and comparison with mass-spectral library data. Sixteen GC–MS features were annotated, including n-hexane, hexanoic acid, 3-methylpentanoic acid, several hexanoic acid esters, phenyl acetate-related material, vinyl acetate, 1,2-ethanediol diacetate, eicosyl acetate, octadecyl

acetate, eicosanoic acid eicosyl ester, an oleate ester, octan-2-yl 2-methylbutanoate, an oxalate ester, 3-octanol acetate and a dimethoxy-dimethyl octanol derivative.

2.11 LC–MS analysis

For LC–MS analysis, 1 g of dried extract was extracted with 20 mL of 80:20 methanol/water. The mixture was vortexed and sonicated for 15 min, followed by centrifugation at 4,000 rpm for 10 min. Formic acid and sodium hydroxide were used as described in the original procedure, after which the preparation was defatted with hexane, passed through a 0.22-µm PTFE membrane and diluted for analysis. Separation was performed on an Agilent 1260 Infinity II LC system. Ten constituents were tentatively assigned from the LC–MS data: coumarin, nornicotine, nicotine, caffeic acid, scopoletin, anabasine, kaempferol, quercetin, aesculin and chlorogenic acid. Their reported relative chromatographic areas were 6.681%, 18.811%, 8.366%, 11.820%, 7.604%, 7.376%, 8.358%, 6.859%, 10.020% and 14.101%, respectively.

2.12 Statistical analysis

Results were expressed as mean ± standard error of the mean. Dunnett's t-test was used for statistical comparisons, and $p < 0.05$ was regarded as statistically significant.

3. Results

3.1 Extraction yield

Table 4.1.1 Percentage Yield of Cold ethanol maceration of 200 g of dried *N. tabacum* leaves produced

Extract	Yield (g)	Yield (%w/w)
Ethanol	l8.95	2.24

3.2 Preliminary phytochemical profile

Qualitative screening showed that the ethanol extract contained alkaloids, flavonoids, cyanogenic glycosides, carbohydrates, phenolic constituents and steroid/triterpenoid-related compounds. No evidence of anthraquinones, saponins, phlobatannins, cardiac glycosides or fixed oils was obtained under the conditions of the tests.

3.3 Antibacterial activity of the crude ethanol extract

Table 4.1.3: Antimicrobial Assay (Zone of inhibition "mm") of the Crude ethanol extract of *N. tabaccum*

Fractions Conc. (*µg/L*)	*P. aeruginosa*	*E. coli*	*Klebsiella spp.*	*Staph. aureus*	*Proteus spp.*	*C. albicans*
100	20.00 ± 2.646	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50.0	16.33 ± 2.517	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25.0	13.33 ± 1.528	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12.5	11.33 ± 1.528	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6.25	9.67 ± 1.528	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethanol	0.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ciprofloxacin	2.00 ± 0.20	1.67 ± 0.00	6.67 ± 0.00	3.33 ± 0.00	5.00 ± 0.00	--
Fluconazole	--	--	--	--	--	2.50 ± 0.20

The mean difference is significant at $p < 0.05$ level; n = 3

3.4 Activity of chromatographic fractions

Table 4.1.4: Antimicrobial Assay (Zone of inhibition "mm") of the fractions of ethanol extract of *N. tabaccum*

Fractions Conc. (*µg/L*)	*P. aeruginosa*	*E. coli*	*Klebsiella spp.*	*Staph. aureus*	*Proteus spp.*	*C. albicans*
Fraction 1	4.00 ± 0.44	2.00 ± 2.00	2.00 ± 2.00	2.60 ± 2.60	2.00 ± 2.00	4.60 ± 3.03
Fraction 2	2.00 ± 2.00*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fraction 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fraction 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fraction 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ciprofloxacin	2.00 ± 0.20	1.67 ± 0.00	6.67 ± 0.00	3.33 ± 0.00	5.00 ± 0.00	--
Fluconazole	--	--	--	--	--	2.50 ± 0.20

3.5 Elemental composition

The reported concentrations were 0.015 mg/L for Pb, less than 0.001 mg/L for As, 0.06 mg/L for Mn and 0.0023 mg/L for Cd; Hg, Co, Ni and Cu were not detected.

Table 4.1.5: Heavy Metals Analysis of ethanol extract of *N. tabaccum*

Metals	Concentrations mg/L	WHO Standard (mg/L)
Lead	0.015	0.015– 0.015 mg/L
Arsenic	< 0.001	≤0.01 mg/L
Manganese	0.06	0.5
Cadmium	0.0023	**Adult**: 2.2 – 12 µg/kg bwt /month **Children** (0.5 – 12 year): 3.9 – 20.6 µg/kg bw./month
Mercury	ND	-
Cobalt	ND	-
Nickel	ND	-
Cooper	ND	-

ND= Not Detected

3.6 GC–MS chemical profile

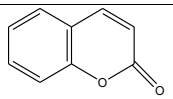
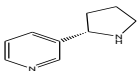
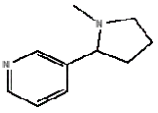
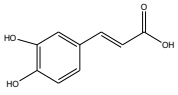
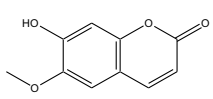
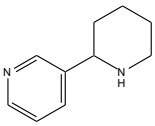
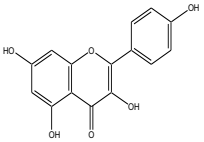
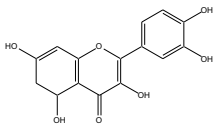
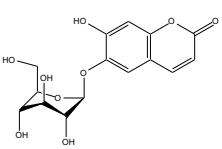
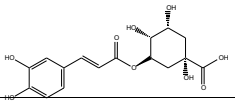
Table 4.1.6: GC-MS compounds of ethanol extract of *N. tabaccum*

S/N	Retention Time (min)	Name of Compound	Molecular Formula	Molecular Weight(Å)	Nature of the compound	% Composition	Uses/Activity	Reference(s)
1	3.66	n-Hexane	C_6H_{14}	86	Ester derivatives of fatty acids	1.261	As a solvent	
2	5.079	Hexanoic Acid	$C_6H_{12}O_2$	116	Ester derivatives of fatty acids	1.354	Antimicrobial Anti- inflammatory	Chebouat *et al*., 2016; Aranega *et al*., 2014.
3	7.832	Pentanoic Acid, 3-methyl	$C_6H_{12}O_2$	116	Ester derivatives of fatty acids	6.830	Antibacterial activity	Budayatin *et al*., 2021.
4	7.872	Hexanoic acid, 1-methylethyl ester	$C_9H_{18}O_2$	158	Ester derivatives of fatty acids	7.924	Antibacterial Properties Inflammation Modulation	Rosselli *et al*., 2007.
5	7.958	Hexanoic acid, 1,1-dimethylethyl ester	$C_{10}H_{20}O_2$	172	Ester derivatives of fatty acids	9.671	**Antimicrobial** Solvent Properties	**Ren *et al*., 2019.**
6	8.038	Phenol, 3-ethyl-acetate	$C_{10}H_{12}O_2$	164	Ester derivatives of fatty acids	0.745	Antimicrobial Anti-inflammatory	Byeon *et al*., 2008; Shih *et al*., 2013.
7	8.181	Acetic acid, ethenyl ester	$C_4H_6O_2$	86	Ester derivatives of fatty acids	3.218	Potential Antimicrobial Solvent	
8	8.261	1,2-ethanediol, diacetate	$C_6H_{10}O_4$	146	Ester derivatives of fatty acids	7.363	Anti-fungal Solvent	Ebadzadsahrai *et al*., 2020.
9	9.651	Eicosyl acetate	$C_{22}H_{44}O_2$	340	Ester derivatives of fatty acids	3.209	Anti-inflammatory Antimicrobial	Elwekee *et al*., 2023; Nogueira *et al*., 2008.
10	9.691	Acetic acid n-octadecyl ester	$C_{20}H_{40}O_2$	312	Ester derivatives of fatty acids	4.832	Antioxidant	Ajiboye *et al*., 2015.
11	9.754	Eicosanoic acid, eicosyl ester	$C_{40}H_{80}O_2$	592	Ester derivatives of fatty acids	2.238	Antimicrobial Potential Role in Drug Delivery	Sosa *et al*., 2016.
12	9.874	Oleic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	592	Ester derivatives of fatty acids	2.199	Anti-inflammatory and Antioxidant	Diab *et al*., 2021
13	10.189	Octan-2-yl 2-methylbutanoate	$C_{13}H_{26}O_2$	214	Ester derivatives of fatty acids	6.955	Potential Anti-inflammatory Effects	
14	10.258	Oxalic acid, decyl 2-ethylhexyl ester	$C_{20}H_{38}O_4$	342	Ester derivatives of fatty acids	1.636	Not available	
15	10.401	3-Octanol acetate	$C_{10}H_{20}O_2$	172	Ester derivatives of fatty acids	0.990	Potential Antimicrobial ActivityChemical Intermediate	Susana *et al*., 2011
16	10.681	2-Octanol B, B-dimethoxy-2,6-dimethyl	$C_{12}H_{26}O_3$	218	Ester derivatives of fatty acids	2.576	Anti-inflammatory Potential Antimicrobial Properties	Najda *et al*., 2021.

3.7 LC–MS chemical profile

The LC–MS results showed a mixture of nitrogen-containing alkaloids together with oxygenated metabolites belonging to phenolic, flavonoid and coumarin-related groups.

Table 4.1.7: Result of LC-MS compounds of ethanol extract of *N. tabaccum*

S/N	Name Of Compound	Structure	Molecular Formulae	Molecular Weight (Å)	Retention Time	% Total Area	Biological Uses	Reference(s)
1	Coumarin		$C_9H_6O_2$	146	4.491	6.681%	Anti-inflammatory. Anti-coagulant. Anti-bacteria, Antifungal. Antiviral. Anticancer.	Srikrishna *et al*., 2016; DellaGreca *et al*., 2003
2	Nornicotine		$C_9H_{12}N_2$	148.209 g·mol^{-1}	6.814	18.811%	Cardiovascular effect	Stairs *et al*., 2006
3	Nicotine		$C_{10}H_{14}N_2$	162	6.871	8.366%	Anti-inflammatory, Vascular dysfunction, proliferation, inhibiting apoptosis, neoangiogenesis	Cardinale *et al*., 2012; Catassi *et al*., 2008.
4	Caffeic Acid		$C_9H_8O_4$	180	6.917	11.820%	Anti-inflammatory. Antiviral, Obesity. Anti-bacteria. Alzheimer's disease. Athletic performance, antioxidant	Genaro-Mattos*et al*., 2015; Kaio*et al*., 2019
5	Scopoletin		$C_{10}H_8O_4$	192	7.014	7.604%	Antifungal, antibacterial, antioxidant, anti-inflammatory	Kishore et al., 2017; Mogana*et al*., 2013; Lemos*et al*., 2020.
6	Anabasine		$C_{10}H_{14}N_2$	162	7.100	7.376%	Anti-insecticidal,	Zammit *et al*, 2014
7	Kaempferol		$C_{15}H_{10}O_6$	286	7.580	8.358%	Anti-inflammatory, antimicrobial, antioxidant, analgesic	Neuhouser, 2004; Pei *et al*., 2017; Alam*et al*., 2020.
8	Quercetin		$C_{15}H_{10}O_7$	302	8.336	6.859%	Antioxidant, anti-inflammatory, anti-atherosclerotic, antihypertensive, vasodilator, antiobesity, anti-hypercholesterolemic	Salvamani *et al*., 2014; Sultana & Anwar, 2008.
9	Aesculin		$C_{15}H_{16}O_9$	340	9.034	10.020%	Anti-inflammatory, antibacterial, antioxidant and anti-tumor properties	Rostom *et al*., 2022; Sinha*et al*., 2022.
10	Chlorogenic Acid		$C_{16}H_{18}O_9$	354	10.911	14.101%	Antispasmodic, antioxidant, anti-cancer, anti-obesity	Farah *et al*., 2005; Kweon*et al*., 2001.

4. Discussion

Cold ethanol maceration of the powdered tobacco leaves produced an extract yield of 2.24% w/w. A relatively modest yield does not, on its own, indicate that the plant material lacks pharmacological potential. Ethanol is capable of extracting compounds across a useful range of polarities, including many phenolics, flavonoids and alkaloids as well as some lipid-associated constituents. Extraction efficiency can also be altered by factors such as solvent polarity, duration of extraction, particle size, developmental stage of the plant and drying conditions.[15]

The phytochemical screening further demonstrated that the extract was chemically diverse. The detection of alkaloids, flavonoids and phenolic compounds is consistent with the recognized metabolite richness of *Nicotiana* species, which includes nicotine-related alkaloids, phenylpropanoids, flavonoids, diterpenes and other specialized compounds.[8,10] Previous studies have likewise reported both phytochemical diversity and antimicrobial activity in *N. tabacum* extracts, while demonstrating that the biological response can change substantially with the extraction solvent.[12,13] Thus, extracts obtained from different plant materials or under different extraction conditions should not be assumed to have equivalent chemical or biological properties.

The LC–MS profile offers several possible chemical explanations for the antibacterial response observed in the crude extract. Caffeic acid, chlorogenic acid, kaempferol, quercetin, scopoletin, coumarin and aesculin were among the tentatively assigned compounds, and each belongs to a chemical class with reported biological activity. Their coexistence in the same extract makes a multi-component contribution plausible. However, the present data cannot identify which constituent, if any, is principally responsible for the inhibition, and chromatographic peak area should not be treated as a direct measure of biological potency or absolute concentration. The detection of caffeic and chlorogenic acids is notable because these phenylpropanoid-derived compounds are common plant constituents with recognized antioxidant and anti-inflammatory properties. Studies of *N. tabacum* have also shown active regulation of chlorogenic-acid and flavonoid biosynthesis through the phenylpropanoid pathway.[16] Whether these metabolites contribute directly to the antibacterial effect requires confirmation with purified compounds and controlled dose-response experiments. Scopoletin and coumarin are similarly relevant because coumarin-derived structures have attracted considerable interest in antimicrobial medicinal chemistry. The identification of kaempferol and quercetin is also noteworthy, as flavonoids may affect bacterial membranes, oxidative processes, enzyme systems and virulence-related pathways. The combined presence of these metabolite classes therefore provides a reasonable basis for investigating a mixture-dependent antibacterial effect, but it does not establish causation.

The elemental results provide preliminary information relevant to the safety profile of the extract. Lead, arsenic, manganese and cadmium were detected at the concentrations reported in this study, whereas mercury, cobalt, nickel and copper were not detected. The presence of trace elements in plant materials is important because plants can accumulate substances from their environment, and tobacco has particular relevance in studies of elemental exposure[19] Nevertheless, the concentrations measured here is not being interpreted as definitive evidence that the extract is safe for therapeutic use. Safety assessment would require a broader toxicological programme, especially because the extract also contains tobacco alkaloids with established pharmacological activity. The clinical setting of CSOM further complicates interpretation because persistent infection may involve several organisms and biofilm formation, which can modify antimicrobial susceptibility and contribute to treatment failure.

The antimicrobial results obtained after fractionation showed that the separated fractions were considerably less active than the original crude extract. This pattern raises the possibility that the antibacterial effect may depend on additive or synergistic interactions among constituents, rather than on the activity of one isolated compound alone.

5. Conclusion

The findings of this study indicate that an ethanol extract of *Nicotiana tabacum* L. leaves has selective, concentration-dependent antibacterial activity against the clinically isolated *Pseudomonas aeruginosa* strain associated with ear infection. The extraction yield was 2.24% w/w, and preliminary screening demonstrated the presence of alkaloids, flavonoids, phenolic compounds, cyanogenic glycosides, carbohydrates and steroid/triterpenoid constituents. The crude extract was more active than the chromatographic fractions, suggesting that the observed antibacterial effect may involve interactions among several constituents rather than a single dominant compound. GC–MS generated putative assignments for 16 constituents, while LC–MS tentatively assigned 10 metabolites, including phenolic, flavonoid and coumarin-related compounds together with nicotine, nornicotine and anabasine. Although these findings provide useful chemical leads, the analytical annotations do not establish individual compounds as the cause of antibacterial activity. Elemental analysis detected Pb, As, Mn and Cd at the reported

concentrations, while Hg, Co, Ni and Cu were not detected. These results constitute preliminary compositional information and should not be taken as proof of therapeutic safety. Overall, *N. tabacum* warrants further investigation as a source of antibacterial molecules active against P. aeruginosa, but the present study does not demonstrate that the crude extract is either clinically effective or safe for treating CSOM. Future work should include standardized MIC and MBC determinations, testing across multiple independent clinical isolates, detailed antibiofilm studies, toxicity and ototoxicity assessment, and bioassay-guided isolation of active constituents.

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