



Original Research

Antibacterial and biological evaluation of selected medicinal plants against multi drug resistant bacteria *Streptococcus pneumoniae*

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Abstract

Streptococcus pneumoniae is a prominent respiratory infection associated with rising antibiotic resistance, necessitating the quest for new bioactive sources. Phenolic and flavonoid chemicals with potential biological action have been found in a number of medicinal plants that have historically been used to treat respiratory and throat-related conditions; nevertheless, there has been little experimental confirmation against *S. pneumoniae*. The purpose of this study was to examine the phytochemical composition, antioxidant capacity, and in vitro antibacterial activity of nine ethnomedicinal plants that have historically been linked to respiratory health. Ethanolic extracts of *Sisymbrium officinale*, *Syzygium aromaticum*, *Fumaria officinalis*, *Hyssopus officinalis*, *Lepidium sativum*, *Strychnos nux-vomica*, *Ephedra gerardiana*, *Cinnamomum zeylanicum*, and *Myristica fragrans* were prepared and evaluated for total phenolic content (TPC) and total flavonoid content (TFC). The DPPH radical-scavenging test was used to measure antioxidant activity, and the agar-well diffusion method was used to screen for antibacterial activity against *S. pneumoniae*. The reference control was ciprofloxacin (5 µg/mL). The results were statistically compared and analyzed as mean ± SD. The phytochemical content and extract yield varied significantly. *Syzygium aromaticum* and *Cinnamomum zeylanicum* exhibited relatively higher TPC and stronger DPPH activity, whereas *Ephedra gerardiana* and *Hyssopus officinalis* produced comparatively larger inhibition zones against *S. pneumoniae* in agar diffusion screening. Ciprofloxacin produced greater inhibitory activity than all extracts, as expected. Moderate correlations were observed between TPC and antioxidant activity. The findings provide preliminary in vitro evidence supporting the biological relevance of selected traditionally used plants; however, they do not confirm therapeutic efficacy. The study is limited by the use of a single antioxidant assay and the absence of MIC/MBC determination and susceptibility profiling of the bacterial strain. Future research should include broth microdilution MIC/MBC testing, cytotoxicity evaluation, bioactive compound characterization, and in vivo validation to substantiate pharmacological potential.

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Introduction: It has often been determined that one of the main threats to global health is infectious illnesses. Medical practitioners have long used plants that have therapeutic benefits. Studies based on evidence have been conducted all around the world to confirm the effectiveness of medicinal plants, and some of these scraps of evidence have shed light on how to synthesize plant-based chemicals that have therapeutic applications¹.

According to their historical use in many cultures, "traditional or herbal medicines" are those derived from plant sources that are usually regarded as safe at the formulation dosage². The World Health Organization (WHO) defines traditional medicine as the entirety of knowledge, abilities, and techniques derived from indigenous ideas, opinions, and experiences from various cultures, whether or not they can be explained. It can be applied to both the preservation of health and the evasion, diagnosis, improvement, or cure for both mental and physical illnesses. In order to determine the antimicrobial activity of medicinal plants, phytochemical screening is typically conducted against a wide range of microorganisms. This screening relies on the active ingredients of the plants, which are mostly secondary metabolites. The majority of bacteria now exhibit resistance to antimicrobial drugs, which has created a major issue³ this made ongoing research for safer and more effective treatment compounds necessary.⁴

Respiratory tract infections caused by *Streptococcus pneumoniae* remain a major global public health concern, particularly due to the increasing incidence of antimicrobial resistance. *S. pneumoniae* is associated with pneumonia, meningitis, septicemia, and otitis media, and therapeutic failure is frequently reported when resistance develops to commonly prescribed antibiotics such as penicillins, macrolides, and fluoroquinolones (Cherazard et al., 2017; Li et al., 2023). The emergence of resistant clinical strains has renewed scientific interest in medicinal plants as potential complementary sources of antimicrobial compounds⁵.

The recent development of bacteria that are resistant to several drugs has limited the availability and cost of various antibiotics that are now prescribed globally⁶. It thus lowers the efficacy of therapeutic strategies and raises the expense, morbidity and mortality of medical care. The increasing incidence of diseases and the expansion of scientific understanding regarding herbal medicines as significant substitutes or supplemental treatments for diseases justify the use of therapeutic substances obtained from plants⁷.

Traditional medicinal systems across different cultures have historically used a wide range of plants to manage respiratory ailments such as cough, sore throat, chest infections, bronchial irritation, and fever. Many of these plants are reported to possess bioactive phytochemicals, including phenolics and flavonoids, which may contribute to antioxidant and antimicrobial properties. However, for most traditionally used plants, systematic laboratory validation of their biological activity against respiratory pathogens remains limited⁸.

Numerous investigations have revealed the presence of coumarins, flavonoids, phenolics, tannins, alkaloids,

terpenoids, essential oils, polypeptides, and polyacetylenes in plants used for medicine. These bioactive substances serve as building blocks for the production of antibiotics, which are administered for treating infectious illnesses⁹. Certain plants may possess broad-spectrum antimicrobial properties, potentially mitigating the negative consequences linked to microorganisms resistant to many drugs¹⁰. Given that over 80% of people worldwide rely on conventional medicine for their basic medical care, it has been noted that chemical production and the hunt for natural products from living organisms are the main sources of discovery for a new bioactive substance to treat human illness caused by pathogenic microbes¹¹. Because of this, traditional medicinal plants (TMP) are extremely significant source of novel bioactive chemical compounds because of their unique chemical abundance within each species and ecological diversification.

Accordingly, the *Sisymbrium officinale*, *Syzygium aromaticum*, *Fumaria officinalis*, *Hyssopus officinalis*, *Lepidium sativum*, *Strychnos nux-vomica*, *Ephedra gerardiana*, *Cinnamomum zeylanicum*, and *Myristica fragrans* are TMP utilized by societies for the cure of various ailments instigated by notorious contagious agents. However, there haven't been many in-depth analyses of these plants' potential as antimicrobials or phytochemical entities acting as therapeutic agents against the multidrug-resistant bacteria *Streptococcus pneumoniae*¹².

Sisymbrium officinale (Hedge mustard), is a member of the *Brassicaceae* family. Traditionally, its aerial parts have been utilized as a treatment for respiratory tract ailments like asthma, pharyngitis, laryngitis, coughs, aphonia, common colds, and sore throats¹³. A study indicated that the *Sisymbrium officinale*'s extract exhibited strong antimicrobial action counter to both *E. coli* and *Streptococcus* bacteria¹⁴.

Syzygium aromaticum (L.) (Clove), is a member of the *Myrtaceae* family. It is a popular food taste that has historically been used to heal headache, sore throat, respiratory issues, and dental issues. Its hypotensive, antibacterial, and antidiabetic qualities have been extensively described in Nigerian literature¹⁵.

Fumaria officinalis L. (Shahterah) belongs to *Fumariaceae* family. Previous research in the field of pharmacology demonstrated the benefits of *Fumaria officinalis* for biliary problems, irritable bowel syndrome, and neural, analgesic, antioxidant, anticancer, antibacterial, antidiabetic, and aphrodisiac effects¹⁶.

Hyssopus officinalis (Zufah) is an herb that is often grown in Iran and is a flowering plant in the *Lamiaceae* family. Indigenous medicine has utilized this plant for a number of purposes, including anti-inflammatory, anti-bacterial, antipyretic, anti-spasmodic, antihypertensive, and anti-hyperlipidemic effects¹⁷.

Lepidium sativum L., (garden cress), is a member of the *Brassicaceae* family. The pharmacological actions of this plant are diverse and include antibacterial, antifungal, antioxidant, cytotoxic, diuretic, hepatoprotective, hypoglycemic, antiosteoporotic, antiasthmatic, anti-carcinogenic, cardiostimulant, smooth and skeletal muscle

contraction, fracture healing, chemoprotective effects, and hemagglutinating activity^{18 19}.

Strychnos-nux-vomica (Kuchla), a member of the *Loganiaceae* family. Although this plant's potential medicinal use has not been discovered by modern medicine, traditional Ayurveda medicine acknowledges it as a therapeutic agent following a unique detoxifying process known as "Sodhan karma". Its anticonvulsant, antitumor, anti-amnesic, analgesic, anti-inflammatory, immune-modulatory, anti-snake venom, hepatoprotective, anti-diarrheal, and anti-cholestatic properties have been documented in the literature²⁰.

Commonly referred to as "Ma-huang", *Ephedra gerardiana* is a native of the Himalayan region and is used medicinally. For many years, its bronchodilatory, diaphoretic, diuretic, and thermogenic qualities have been utilized in conventional medicine. It has additionally been employed as a stimulant for treating edema, fever, allergies, headaches, bronchial asthma, chills, colds, coughs, flu, and nasal congestion^{21 22}.

Cinnamomum zeylanicum, often known as the real cinnamon, is one of the members of the *Lauraceae* family. Treatments for dyspeptic illnesses, flatulence, decreased appetite, stomach discomfort, diarrhea, eye inflammation, leucorrhoea, vaginitis, rheumatism, neuralgia, wounds, and toothaches embrace *C. zeylanicum*. The anti-inflammatory, antibacterial, antimicrobial, anticancer, cardiovascular, cholesterol-lowering, immunomodulatory, larvicidal, and antioxidant activities of cinnamon have all been noted²³.

Myristica fragrans Houtt. In India, it is also known as Jaiphal and Javitri, and it is a member of the *Myristicaceae* family. It produces mace and nutmeg, two spices. Recent research has revealed that *M. fragrans* contains anti-inflammatory, anticonvulsant, analgesic, antioxidant, antibacterial, and antifungal properties²⁴.

The nine plants selected in this study were chosen based on Documented ethnomedicinal use related to respiratory or throat conditions, including cough, bronchial irritation, asthma-like symptoms, fever, or throat infections. Traditional relevance in Unani, Ayurvedic, and folk medicine for treating conditions associated with respiratory tract infections. Previous pharmacological reports indicating antioxidant, antimicrobial, or related biological activities that may support respiratory health²⁵. This study therefore hypothesized that plants traditionally used for respiratory ailments may exhibit measurable antioxidant activity and inhibitory effects against *S. pneumoniae*, a major respiratory pathogen²⁶.

In earlier versions of this manuscript, the strain was described as "multidrug-resistant (MDR)." However, as susceptibility profiling data were not included, this study refers to the test organism simply as *Streptococcus pneumoniae*. The potential resistance profile of the strain is acknowledged as a limitation and will be addressed in future investigations.

The present research aimed to determine the total phenolic content (TPC) and total flavonoid content (TFC) of ethanolic extracts of selected medicinal plants, evaluate their antioxidant potential using the DPPH radical scavenging assay and assess antibacterial activity against *S. pneumoniae* using the agar-well diffusion method.

The results are not meant to serve as final therapeutic confirmation, but rather as initial screening data.

To support pharmaceutical uses, more research will be needed, including MIC/MBC determination, cytotoxicity testing, and mechanistic review.

MATERIALS AND METHODS:

Plant selection and ethnopharmacological basis: Based on ethnomedicinal literature, historical use in Unani and Ayurvedic practice, and earlier studies of biological or antimicrobial relevance, nine medicinal plants that are historically used for respiratory or throat-related diseases were chosen. The selected plants included *Sisymbrium officinale*, *Syzygium aromaticum*, *Fumaria officinalis*, *Hyssopus officinalis*, *Lepidium sativum*, *Strychnos nux-vomica*, *Ephedra gerardiana*, *Cinnamomum zeylanicum*, and *Myristica fragrans*. The selection criteria are aligned with their documented traditional use in conditions associated with respiratory infections.

Collection, identification, and processing of plant material: Between March and June of 2024, plant materials were gathered from natural sources and verified herbal markets in Pakistan. Voucher specimens were placed in the institutional herbarium for reference once a taxonomist completed the botanical identification. The plant parts that have historically been used medicinally were ground into a powder using a mechanical grinder after being air-dried at room temperature in the shade.

Preparation of plant extracts: Approximately 50 g of powdered plant material was macerated in 500 mL of 96% ethanol for 72 hours, shaking intermittently. A rotary evaporator was used to filter and concentrate the extracts under low pressure. Dry weight was used to compute extract yield (%). To guarantee reproducibility and ease of interpretation, all concentrations reported in this study are given as mg of extract per mL of solvent (mg/mL, dry weight equivalent).

Test Organism: *Streptococcus pneumoniae* was the bacterial strain utilized in this investigation. The strain is not classified as multidrug-resistant (MDR) since antibiotic susceptibility profiling was not carried out as part of this experiment. Resistance characterization will be taken into consideration in subsequent studies, and this restriction is acknowledged.

Antioxidant activity — DPPH radical scavenging assay: The DPPH assay was used in accordance with normal procedure to assess the antioxidant capacity of plant extracts. In summary, extracts were evaluated at doses between 50 and 400 µg/mL. A UV-Vis spectrophotometer was used to detect absorbance at 517 nm, and the percentage of inhibition was computed. The positive control was ascorbic acid. The findings are shown as mean ± SD (n = 3).

Methodological note: Results are viewed as first markers of antioxidant capability because the DPPH test represents a single antioxidant pathway. Complementary assays like ABTS or FRAP may be used in future research to provide a more comprehensive evaluation of antioxidant behavior.

Antibacterial Assay — agar well diffusion method: The antibacterial activity against *S. pneumoniae* was assessed using the agar-well diffusion technique. At doses of 25, 50, and 100 mg/mL, extracts were evaluated. 100 µL

of the extract solution was added to wells with a diameter of 6 mm. Zones of inhibition (mm) were measured after plates were incubated for 24 hours at 37 °C.

Positive and negative controls:

- **Positive control:** Ciprofloxacin at 5 µg/mL, prepared according to CLSI guidelines.
- **Negative control:** Solvent control (ethanol).

To improve interpretability, inhibition zones of extracts were qualitatively compared with ciprofloxacin; however, no direct equivalence is assumed due to differences in molecular diffusion characteristics.

Methodological limitation: A semi-quantitative screening technique that is impacted by extract diffusibility is agar diffusion. In order to facilitate more thorough potency comparison, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values will be calculated in subsequent research.

Phytochemical analysis: Gallic Acid Equivalent (GAE)/g extract and Quercetin Equivalent (QE)/g extract, respectively, were used to quantify and express the total phenolic content (TPC) and total flavonoid content (TFC). For reproducibility and clarity, units are constantly included in all tables and figures.

Statistical analysis: Every experiment was carried out in triplicate, and the mean ± standard deviation (SD) is used to express the results. One-way ANOVA was used to examine the data, and then the relevant post-hoc comparisons were made. At $p < 0.05$, statistical significance was taken into consideration. To help interpret reported patterns, correlation analyses between TPC/TFC and biological activities were carried out when appropriate.

Extract yield and phytochemical composition: The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts are presented in Table 1, expressed as mg GAE/g extract and mg QE/g extract, respectively. *Cinnamomum zeylanicum* and *Syzygium aromaticum* exhibited comparatively higher TPC values, while *Hyssopus officinalis* and *Ephedra gerardiana* showed elevated TFC. A summary of extract yield, TPC, and TFC values is provided with units clearly stated in all column headings, and a descriptive table legend is included for clarity. In this study, the total phenolic content of the ethanolic extracts of particular 9 medicinal plants were determined. Moreover, the Folin-Ciocalteu method (Folin and Ciocalteu, 1927) was employed to study it, and gallic acid was used as the standard. Gallic Acid Equivalents (mg of GAE/g of extract) are the unit of measurement used to express the results. Four different concentrations 80, 40, 20 and 10 mg/mL of ethanolic extract were used to determine total phenolic content of each plant.

Total phenolic contents (TPC) of the ethanolic extract of selected medicinal plants: In this study, *Ephedra gerardiana* has highest total phenolic content measuring 535.6 mg GAE/g in 80 mg/mL concentration of ethanolic extract among all tested medicinal plants. While the *Sisymbrium officinale* has lowest total phenolic content measuring 365.2 mg GAE/g in 80 mg/mL concentration of ethanolic extract among all tested medicinal plants. With 40 mg/mL ethanolic extract concentration, among all investigated medicinal plants *Ephedra gerardiana* showed highest TPC value 485.800 mg GAE/g while *Myristica*

fragrans indicated to possess lowest TPC range 330.600 mg GAE/g. With 20 mg/mL ethanolic extract concentration, among all tested medicinal plants *Syzygium aromaticum* has highest TPC value 464 mg GAE/g while *Myristica fragrans* showed lowest TPC value 310.4 mg GAE/g. With 10 mg/mL ethanolic extract concentration, among all tested medicinal plants *Strychnos nux-vomica* indicated to have highest TPC value 422.2 mg GAE/g while *Hyssopus officinalis* showed lowest TPC value 291.800 mg GAE/g.

These results show that the plants ethanolic extracts at different concentrations are rich in polyphenolic compounds.

Total Flavonoid Contents (TFC): By the aluminium chloride colorimetric technique, total flavonoid content was found out. Total flavonoid contents (TFC) of the extracts were expressed as µg catechin equivalents per mL of plants extract from the linear regression curve of catechin. Four different concentrations 80, 40, 20 and 10 mg/mL of ethanolic extract were used to determine total flavonoid content of each plant. In all four ethanolic extract concentrations (80, 40, 20, 10 mg/mL) *Ephedra gerardiana* indicated to have highest TFC values while *Myristica fragrans* showed lowest TFC values respectively, among all tested medicinal plants.

Antioxidant activity (DPPH Assay): The antioxidant potential of the extracts, measured using the DPPH radical scavenging assay, is shown in Table 2 and Figure 1. Results are presented as mean ± SD (n = 3) for extract concentrations ranging from 50–400 µg/mL.

A concentration-dependent increase in antioxidant activity was observed for all extracts. The highest DPPH scavenging activity was recorded for *Syzygium aromaticum* and *Cinnamomum zeylanicum*, whereas *Strychnos nux-vomica* showed comparatively weaker activity. Ascorbic acid, included as a reference antioxidant, demonstrated the greatest percentage inhibition across all concentrations, as expected.

Interpretation note: Since the DPPH assay represents a single antioxidant mechanism, these findings should be considered preliminary. Additional assays (e.g., ABTS, FRAP) will be required to provide a broader evaluation of antioxidant capacity.

In this investigation, *Hyssopus officinalis* revealed to possess highest percentage inhibition of DPPH valuing 66.339% with 80 mg/mL ethanolic extract concentration, while *Myristica fragrans* showed lowest percentage inhibition of DPPH valuing 43.359% with 80 mg/mL ethanolic extract concentration. In this study, among all tested medicinal plants, in all four ethanolic extract concentrations (80, 40, 20, 10 mg/mL) *Myristica fragrans* indicated to possess lowest percentage inhibition of DPPH ranging 43.359, 38.721, 34.926, and 31.483 percent, respectively.

While *Hyssopus officinalis* has highest % inhibition of DPPH with 80 and 40 mg/mL ethanolic extract concentrations, among all tested medicinal plants. With 20 and 10 mg/mL ethanolic extract concentrations, among all studied medicinal plants *Lepidium sativum* showed highest percentage inhibition of DPPH ranging 57.063 and 53.268 percent, respectively.

Flavonoids and phenolic components are probably the sources of antioxidants in the selected medicinal plants that are responsible for the antioxidant activity by hydrogen transfer methods, based on the study's findings of the scavenging activity of ethanolic extract from selected medicinal plants. The results of the present study demonstrate that the percentage inhibition (free radical scavenging activity) of the plant extract rises with the number of flavonoids present, demonstrating a stronger potential for antioxidants. *Myristica fragrans*, one of the medicinal plants studied for this research, has the lowest flavonoid concentration and the lowest percentage of free radical generation inhibition, making its extract the lowest antioxidant-rich.

Antibacterial Activity Against *Streptococcus pneumoniae*: The antibacterial effects of the plant extracts against *S. pneumoniae* were evaluated using the agar-well diffusion method. Zones of inhibition (mm) at extract concentrations of 25, 50, and 100 mg/mL are presented in Table 3 and Figure 2, reported as mean \pm SD (n = 3).

Among the tested extracts, *Ephedra gerardiana* and *Hyssopus officinalis* produced the largest inhibition zones at higher concentrations, indicating comparatively stronger antibacterial effects under the current experimental conditions. Other extracts produced moderate to weak inhibitory activity.

Comparison with Ciprofloxacin (Reference Control): Ciprofloxacin (5 μ g/mL) produced a substantially larger inhibition zone than all plant extracts, consistent with its established clinical potency. Comparisons between ciprofloxacin and plant extracts are interpreted qualitatively only, because diffusion behavior differs between purified antibiotics and complex plant extracts, and no normalization by active compound concentration was attempted.

Methodological clarification: MIC and MBC values were not determined in this preliminary screening; therefore, antibacterial strength cannot be quantitatively compared with standard antibiotics. This limitation is acknowledged and will be addressed in future work.

While the zone of inhibition of ciprofloxacin is 37mm which was used as standard antibiotic in this study. According to these findings, the ethanolic extracts of selected medicinal plants have good antibacterial potential against *Streptococcus pneumoniae*, so these plants can be considered to be used to combat the bacterial infections.

Statistical and correlation analysis: One-way ANOVA revealed statistically significant differences ($p < 0.05$) among extracts and concentrations for both antioxidant and antibacterial measurements. Post-hoc comparisons indicated that extracts with higher TPC/TFC generally exhibited stronger DPPH activity. A moderate positive correlation was observed between TPC and antioxidant activity, and a weaker trend was noted between TFC and antibacterial activity; however, these associations did not demonstrate a direct causal relationship and should be interpreted cautiously. Further targeted compound-specific analysis is required to validate these trends.

Discussion: The present study evaluated the phytochemical composition, antioxidant potential, and antibacterial activity of nine medicinal plants traditionally

employed for respiratory and throat-related ailments. While the findings provide preliminary laboratory-based evidence supporting the ethnomedicinal relevance of several species, important methodological and interpretive limitations must be acknowledged^{27,28}.

Variations observed in extract yield, total phenolic content (TPC), and total flavonoid content (TFC) reflect intrinsic differences in phytochemical composition among plant species as well as differences in solvent affinity and extractability. Consistent with previous reports, *Syzygium aromaticum* and *Cinnamomum zeylanicum* exhibited relatively higher phenolic content, likely attributable to their richness in phenolic acids and essential oil constituents.^{29,30} Phenolic and flavonoid compounds are widely associated with antioxidant and antibacterial properties, which may partly explain the enhanced biological activity observed in these extracts.³¹ However, it is important to emphasize that TPC and TFC represent non-specific screening indices, and without compound-level identification, no definitive conclusions can be drawn regarding the direct contribution of phenolics to bioactivity.

Antioxidant activity and its interpretation: All tested extracts demonstrated concentration-dependent DPPH radical scavenging activity, with *Syzygium aromaticum* and *Cinnamomum zeylanicum* showing comparatively higher antioxidant effects. These observations align with previous studies reporting strong antioxidant potential in phenolic-rich aromatic plants^{32,28}.

Nevertheless, the DPPH assay evaluates only a single mechanism of free-radical scavenging and does not accurately replicate antioxidant behavior under biological conditions³³. Therefore, the antioxidant activity reported herein should be regarded as preliminary in vitro evidence. A more comprehensive evaluation would require additional chemical assays such as ABTS and FRAP, as well as cell-based antioxidant models, to better approximate physiological relevance³¹.

Antibacterial activity and methodological Constraints: Agar-well diffusion screening against *Streptococcus pneumoniae* revealed that *Ephedra gerardiana* and *Hyssopus officinalis* produced comparatively larger zones of inhibition. These findings suggest the presence of antibacterial constituents capable of suppressing bacterial growth under in vitro conditions, consistent with earlier reports on these species^{34,35}.

However, agar diffusion assays provide only semi-quantitative information and are strongly influenced by extract diffusibility, viscosity, and molecular size of active constituents³⁶. The absence of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination limits accurate ranking of antibacterial potency and prevents meaningful comparison with standard antibiotics.

Although ciprofloxacin produced substantially larger inhibition zones, as expected, this comparison is interpreted strictly qualitatively and does not imply therapeutic equivalence or substitutability of plant extracts³⁷. Future investigations should incorporate broth microdilution-based MIC/MBC assays, time-kill kinetics,

and bioassay-guided fractionation to identify active compounds and establish clinically relevant potency.

Clarification of bacterial resistance terminology: Earlier versions of this manuscript referred to the test strain as multidrug-resistant (MDR). As antibiotic susceptibility profiling was not conducted in the present study, the organism is appropriately referred to simply as *Streptococcus pneumoniae*. Resistance characterization remains an important objective for future studies, particularly in the context of the global antimicrobial resistance crisis³⁸.

Relationship between phytochemical content and biological activity: Moderate correlations were observed between TPC and antioxidant activity, while weaker trends were noted between TFC and antibacterial effects. Although these relationships are consistent with general phytochemical–activity patterns reported in herbal pharmacology, they do not establish causality²⁸. Plant extracts represent chemically complex systems, and biological effects may result from synergistic or antagonistic interactions among multiple constituents. Targeted metabolomic profiling and compound-specific bioassays are therefore required to validate these associations³¹.

Implications and scope of findings: Overall, the findings provide supportive early-stage evidence that selected medicinal plants traditionally used for respiratory conditions possess measurable antioxidant and antibacterial activities relevant to respiratory pathogen research. However, this study should be regarded as a screening investigation rather than proof of therapeutic efficacy. No cytotoxicity testing, selectivity index evaluation, pharmacokinetic analysis, or in vivo validation was performed; therefore, clinical application cannot be proposed based on the current data³⁹.

Conclusion: This study investigated the phytochemical composition, antioxidant potential, and antibacterial activity of nine medicinal plants traditionally associated with respiratory and throat-related ailments. The results indicate that *Ephedra gerardiana*, *Hyssopus officinalis*, *Syzygium aromaticum*, and *Cinnamomum zeylanicum* exhibit measurable antioxidant and in vitro antibacterial effects against *Streptococcus pneumoniae* under the experimental conditions applied. While these findings provide preliminary scientific support for the ethnomedicinal relevance of the selected plants, they do not constitute evidence of therapeutic efficacy. Methodological limitations—including reliance on a single antioxidant assay, absence of MIC/MBC determination, use of agar diffusion as a semi-quantitative antibacterial method, lack of cytotoxicity testing, and absence of in vivo validation—must be considered. Accordingly, the present work should be viewed as an initial screening study that identifies promising plant candidates for further investigation. Future research should incorporate broth microdilution-based MIC/MBC assays, resistance profiling, cytotoxicity evaluation, bioassay-guided isolation, compound identification, and in vivo experimental models to establish pharmacological relevance and safety.

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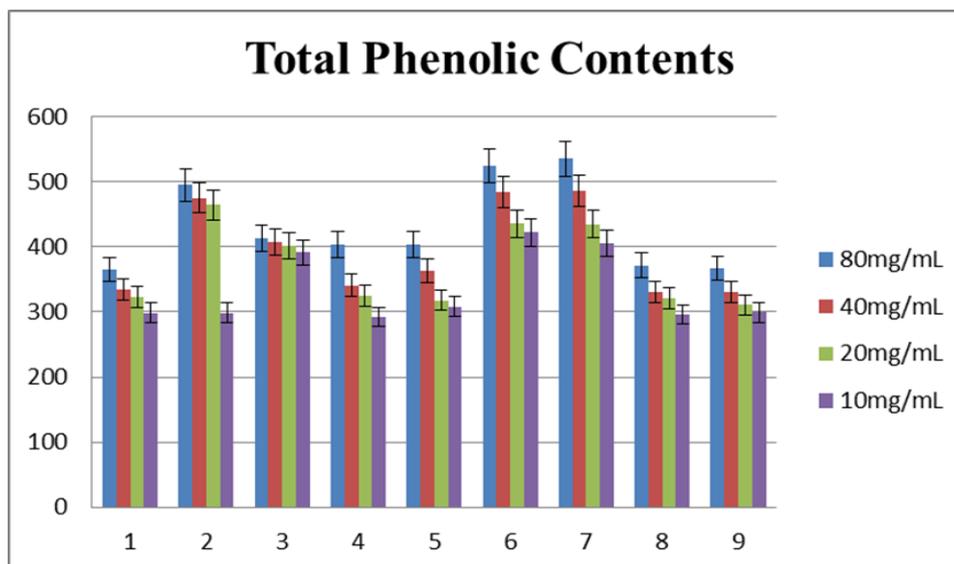


Fig 1. Graphical presentation of total phenolic contents (TPC) of the ethanolic extract of selected medicinal plants

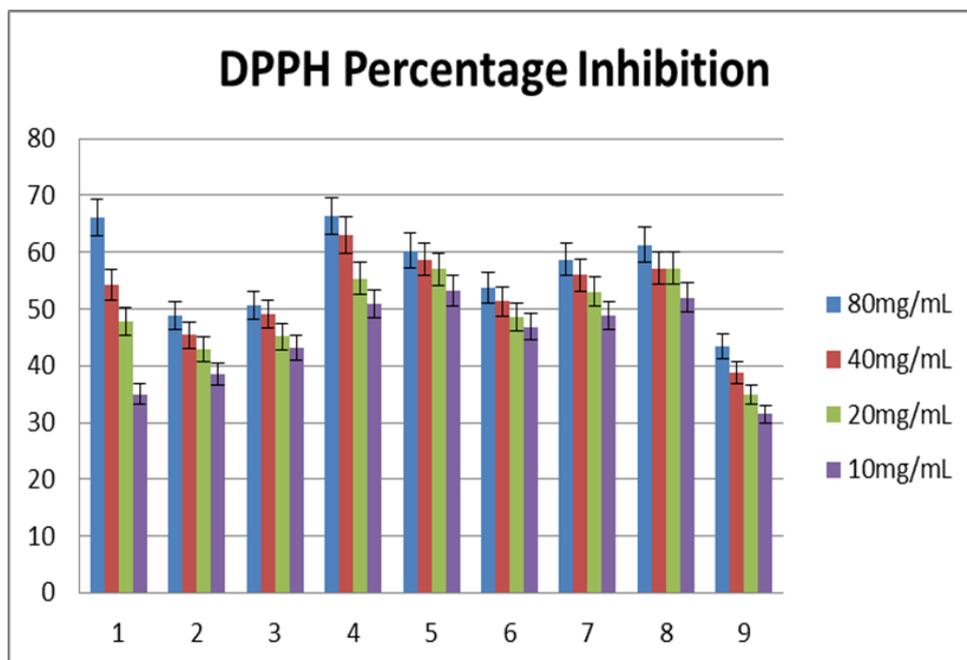


Fig 2. DPPH percentage inhibition



Fig 3. Zone of Inhibition (mm) of 1) *Sisymbrium officinale* 2) *Syzygium aromaticum* 3) *Fumaria officinalis* Plants' Extract showing Antibacterial Activity

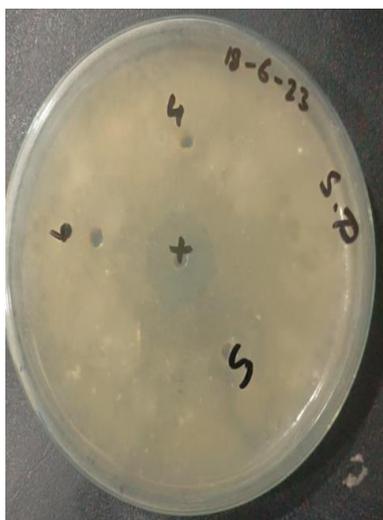


Fig 3.5. Zone of Inhibition (mm) of 4) *Hyssopus officinalis* 5) *Lepidium sativum* 6) *Ephedra gerardiana* Strychnos nux-vomica Plants' Extracts showing Antibacterial Activity

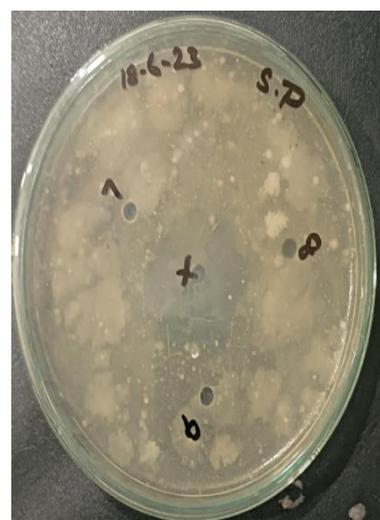


Fig 3.6. Zone of Inhibition (mm) of 7) *Ephedra gerardiana* 8) *Cinnamomum zeylanicum* 9) *Myristica fragrans* Plants' Extracts showing Antibacterial Activity

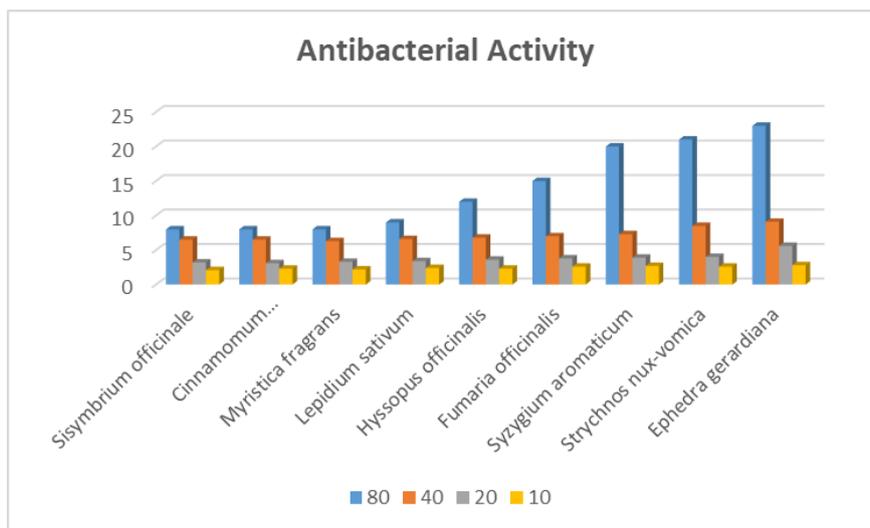


Fig 4. Antibacterial activity

Table 1. Selected Medicinal Plants of the Present Study

| Botanical Name | Local Name | Family | Parts Used |
|------------------------------|-----------------------|---------------|----------------|
| <i>Sisymbrium officinale</i> | Khaksi | Brassicaceae | Seeds |
| <i>Syzygium aromaticum</i> | Long | Mirtaceae | Flowering buds |
| <i>Fumaria officinalis</i> | Shahterah | Fumariaceae | Whole Plant |
| <i>Hyssopus officinalis</i> | Zufah | Lamiaceae | Flowers |
| <i>Lepidium sativum</i> | Hub ul irshad/ Halyon | Brassicaceae | Seeds |
| <i>Strychnos nux-vomica</i> | Kachla | Loganiaceae | Seeds |
| <i>Ephedra gerardiana</i> | Soma Kalpa | Ephedraceae | Whole Plant |
| <i>Cinnamomum zeylanicum</i> | Darchini | Lauraceae | Bark |
| <i>Myristica fragrans</i> | Javitri | Myristicaceae | Seed Covering |

Table 2. Total Phenolic Content (TPC)

| Total Phenolic Content (TPC) (mg of GAE/g of extract) | | | | | | | | | |
|---|----------------------|----------------------|-----------------------|-----------------------|-------------------|----------------------|----------------------|----------------------|--------------------|
| Plant | <i>S. officinale</i> | <i>S. Aromaticum</i> | <i>F. officinalis</i> | <i>H. officinalis</i> | <i>L. sativum</i> | <i>S. nux-vomica</i> | <i>E. gerardiana</i> | <i>C. zeylanicum</i> | <i>M. fragrans</i> |
| 80 | 365.2 | 495.2 | 412.6 | 404.2 | 403.8 | 523.8 | 535.6 | 371.6 | 367 |
| 40 | 333.8 | 475.2 | 407 | 341 | 362.4 | 484.4 | 485.8 | 331 | 330.6 |
| 20 | 322.4 | 464 | 402.2 | 324 | 318 | 435.2 | 435 | 321.2 | 310.4 |
| 10 | 298.8 | 298.8 | 391 | 291.8 | 307.8 | 422.2 | 405.2 | 296.2 | 299.2 |

Table 3. Total Flavonoid Contents (TFC) of the Ethanolic Extract of Selected Medicinal Plants

| Total Flavonoid Content (TFC) (µg catechin equivalents per mL of plants extract) | | | | | | | | | |
|--|----------------------|----------------------|-----------------------|-----------------------|-------------------|----------------------|----------------------|----------------------|--------------------|
| Plant mg/mL | <i>S. officinale</i> | <i>S. Aromaticum</i> | <i>F. officinalis</i> | <i>H. officinalis</i> | <i>L. sativum</i> | <i>S. nux-vomica</i> | <i>E. gerardiana</i> | <i>C. zeylanicum</i> | <i>M. fragrans</i> |
| 80 | 52.368 | 264.211 | 115.263 | 157.895 | 79.737 | 297.105 | 681.053 | 45.789 | 36.579 |
| 40 | 53.421 | 254.737 | 108.947 | 149.474 | 77.105 | 291.842 | 642.895 | 42.632 | 34.474 |
| 20 | 47.368 | 246.842 | 104.211 | 144.737 | 77.895 | 277.895 | 547.105 | 40.789 | 33.158 |
| 10 | 46.579 | 249.211 | 88.421 | 146.842 | 73.684 | 253.684 | 534.211 | 40.789 | 28.158 |

Table 4: Radical Scavenging Activity of the Ethanolic Extract of Selected Medicinal Plants

| DPPH Percentage Inhibition | | | | | | | | | |
|----------------------------|----------------------|----------------------|-----------------------|-----------------------|-------------------|----------------------|----------------------|----------------------|--------------------|
| Plant & Conc. (mg/mL) | <i>S. officinale</i> | <i>S. Aromaticum</i> | <i>F. officinalis</i> | <i>H. officinalis</i> | <i>L. sativum</i> | <i>S. nux-vomica</i> | <i>E. gerardiana</i> | <i>C. zeylanicum</i> | <i>M. fragrans</i> |
| 80 | 66.128 | 48.770 | 50.597 | 66.339 | 60.295 | 53.760 | 58.749 | 61.279 | 43.359 |
| 40 | 54.252 | 45.397 | 49.192 | 62.966 | 58.749 | 51.370 | 56.008 | 57.203 | 38.721 |
| 20 | 47.857 | 42.867 | 45.116 | 55.376 | 57.063 | 48.630 | 53.057 | 52.003 | 34.926 |
| 10 | 34.996 | 38.440 | 43.219 | 50.878 | 53.268 | 46.803 | 48.911 | 38.089 | 31.483 |

Table 5. Antibacterial Activity Against *Streptococcus pneumoniae*

| Medicinal Plant Extract | mm | mm | mm | mm |
|------------------------------|----|-----|-----|-----|
| <i>Sisymbrium officinale</i> | 8 | 6.5 | 3.2 | 2.1 |
| <i>Cinnamomum zeylanicum</i> | 8 | 6.5 | 3.1 | 2.3 |
| <i>Myristica fragrans</i> | 8 | 6.3 | 3.3 | 2.2 |
| <i>Lepidium sativum</i> | 9 | 6.6 | 3.4 | 2.4 |
| <i>Hyssopus officinalis</i> | 12 | 6.8 | 3.6 | 2.3 |
| <i>Fumaria officinalis</i> | 15 | 7 | 3.8 | 2.6 |
| <i>Syzygium aromaticum</i> | 20 | 7.3 | 3.9 | 2.7 |
| <i>Strychnos nux-vomica</i> | 21 | 8.5 | 4 | 2.6 |
| <i>Ephedra gerardiana</i> | 23 | 9.1 | 5.6 | 2.8 |