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In vitro antimicrobial activity of selected herbal extracts against *Mycobacterium tuberculosis*

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Original Article

ABSTRACT

Background: Tuberculosis (TB) is one of the deadliest infectious diseases worldwide, causing approximately two million deaths annually. Multidrug resistance presents a significant global challenge in TB treatment, necessitating prolonged medication use and extended treatment durations. There is an urgent need to discover alternative novel antimicrobial compounds. Herbal medicine offers promising prospects for drug discovery and development, warranting the screening of plant-derived compounds with favorable safety margins.
Objectives: This study aimed to investigate the in vitro antimicrobial activity of extracts from five medicinal plants against *Mycobacterium tuberculosis* in vitro.
Methods: Extracts from five herbs —*Morinda citrifolia* L, *Nicotiana* spp.L, *Centella asiatica* L, *Allium sativum* L, and *Annona muricata* L, —were investigated for their in vitro antimicrobial activity against *M. tuberculosis* using Lowenstein-Jensen medium. Antimicrobial activity was assessed by comparing bacterial colony growth on extract-containing media with that on extract-free control media. The agar dilution method was used to determine antimicrobial activity.
Results: Among the tested extracts, *Nicotiana* spp L and *Annona muricata* L exhibited the strongest antimicrobial activity. These herbs contain various bioactive compounds, including alkaloids, flavonoids, and steroids, which are presumed to contribute to bacterial susceptibility.
Conclusions: The findings suggest that *Nicotiana* spp. L. and *Annona muricata* L. may have therapeutic potential in TB treatment. These herbs could serve as alternative agents for combating TB and may help mitigate the adverse effects associated with standard anti-tuberculosis drugs.

INTRODUCTION

Tuberculosis (TB) is a major global cause of death, with a high prevalence in many developing countries.[1] Based on TB incidence rates, Indonesia ranks third globally after India and China in contributing to the total TB burden.[2] TB is among the top 10 causes of death worldwide and is caused by *Mycobacterium tuberculosis,* which spreads through droplet nuclei.[3-5] Latent TB infection (LTBI) is a major contributor to new TB cases. LTBI refers to individuals who have been infected with the bacteria but do not exhibit symptoms and cannot transmit the disease to others.[6] Approximately 10% of individuals with LTBI will develop active TB, with half of these cases occurring within the first year of infections. Persons with compromised immune systems, such as people living with HIV, experiencing undernutrition, or suffering from diabetes, are at a much higher risk. Several factors contribute to the high incidence of TB, including socioeconomic conditions, cigarette and alcohol consumption, malnutrition, and environmental factors.[5] The World Health Organization (WHO) estimates that approximately 10 million people develop active TB annually, with 90% of cases occurring in adults. Men are more frequently affected than women, and nearly 50% of untreated cases result in death.[7] One of the major challenges in the

TB management is the prevalence of multidrug-resistant TB (MDR-TB), affecting over 50 million people, with approximately 500,000 new drug-resistant cases emerging each year. The irrational use of antibiotics contributes to antimicrobial resistance, leading to treatment failures.[8] TB patients often experience severe side effects due to prolonged treatment durations and the increasing number of prescribed medications.[9]

Herbal medicine has been one of the earliest sources of therapy in healthcare.[10] In recent decades, the use of herbal remedies has grown significantly. Various parts of medicinal plants, including leaves, stems, bark, roots, flowers, and fruits, contain bioactive compounds that contribute to their therapeutic potential. These plant-based compounds can serve as precursors in pharmaceutical drug development or be used in traditional and alternative/complementary medicine for disease prevention and treatment. Medicinal plants may grow naturally in wild terrestrial ecosystems or be cultivated domestically for commercial purposes, providing bioactive compounds for pharmaceutical production.[11]

Herbal medicine is widely recognized in rural areas of many developing countries for both curative and preventive purposes.[12] As a tropical country, Indonesia has an extensive diversity of medicinal plants and herbs, which has been used for generations.[13] Indonesia's forests contain a wide variety of flora, including over 25,000 species of medicinal plants.[14] In daily life, plants play a crucial role in healthcare. Growing concerns about the side effects of modern pharmaceuticals and the increasing need for long-term treatment options have driven interest in herbal medicine, as it is considered safer and more affordable.[15]

Medicinal plants have been identified as promising sources of novel antibiotics.[16] Herbal-based treatments have been widely used to manage various infectious diseases, with potential applications in replacing synthetic drugs.[17] Over recent years, numerous plant species have been evaluated for their effectiveness against *M. tuberculosis*.[18] The growing resistance of M. tuberculosis to conventional drugs and the increasing public interest in medicinal plants with minimal side effects highlight the need for new antimicrobial agents. Efforts to combat TB include technological advancements and collaborative treatment approaches.[9] The resistance challenge posed by *M. tuberculosis* has led to discovery and development of novel antibacterial agents from natural sources. Research is needed to further explore and enhance the potential of herbal plants, which may eventually become valuable therapeutic commodities with multiple benefits.

Commonly used medicinal herbs could serve as an excellent source of novel drugs. This study focuses on investigating the antimicrobial activity of extracts from five medicinal plants commonly used in traditional medicines. Previous research has evaluated the antibacterial properties of noni, tobacco, pegaga, garlic, and soursop extracts against various bacterial pathogens.[19] Given these findings, it is of interest to examine their antibacterial effects against *M. tuberculosis.* Effective TB treatment requires the development of new pharmaceuticals or alternative sources of novel drugs.[20] Considering the diverse therapeutic properties of these plants, this study aims to evaluate their antimicrobial potential against M. tuberculosis at different concentrations and to provide scientific validation for their traditional medicinal uses. Initially, herbs were selected randomly; however, the final five herbs were chosen based on direct ethnopharmacological selection. Previous studies have reported that the antimicrobial properties of these five herbs effectively inhibit pathogenic bacteria.[19] Furthermore, this study seeks to investigate the in vitro antimicrobial activity of herbal extracts from these five plants against *M. tuberculosis*. We also summarize the phytochemical characteristics of these herbs. This research contributes to the exploration of alternative TB treatment modalities in Indonesia.

METHODS

Plant material and extraction

Five plants were selected based on their antimicrobial activities, namely noni (*Morinda citrifolia* L.), tobacco (*Nicotiana* spp., L.), pegaga (*Centella asiatica* (L.) Urb), garlic (*Allium sativum*), and soursop (*Annona muricata* L.). The plant materials were randomly collected from various areas in Central Java, Indonesia. Different plant parts were used; *Morinda citrifolia* L

(Fruit), *Nicotiana* spp., L (Leaf), *Centella asiatica* L (Leaf), *Allium sativum* L (Bulb/Cloves), *Annona muricata* L (Leaf).

The moisture content of herbal materials was measured using a moisture balance device before drying. The materials were shade-dried at room temperature for five days, then ground into coarse powder for extraction and stored in airtight containers. The powdered herbs (100 gr) were macerated with 96% ethanol (1000 ml) at 37°C for one week. The supernatant was filtered through Whatman No. 1 filter paper using a Buchner funnel under vacuum. The resulting filtrate was concentrated by vacuum evaporation with a rotary evaporator at 45°C.[21] The extract was then freeze-dried to obtain an ethanol-free powder, which was further concentrated to dryness under reduced pressure to yield crude extracts. Aqueous extracts were also dried under reduced pressure using a freeze-drier. All crude extracts were stored in the dark at 10°C until further use. The ethanol extract of the herbs underwent qualitative phytochemical screening.[22] Each herbal extract was carefully pipetted into a sterile McCartney bottle, arranged on a specific rack at 30◦ angle, and incubated at 85°C for 45 minutes to promote coagulation. Following incubation, the LJ-medium bottles were kept at room temperature for 24 hours before being inoculated with *M. tuberculosis* isolates. The antibacterial effects of different concentrations (25%, 50%, 75%, and 100%) were evaluated against *M. tuberculosis*.

Laboratory equipment was decontaminated using chemical disinfectants, autoclaving, incineration, or other validated methods. All potentially infectious materials were chemically disinfected prior to disposal and placed in durable, leak-proof containers during collection, handling, processing, storage, and transport within a secured facility. Laboratory personnel were thoroughly trained in the safe handling of infectious materials.

Phytochemical screening methods

Preliminary tests were performed to identify the presence of bioactive compounds in the extract using standard phytochemical screening methods. The analysis revealed the presence of tannins, saponins, flavonoids, steroids, phenolics, triterpenoids, and alkaloids. Tannins were detected by mixing 5 mg of the aqueous extract with 2 mL of a 2% ferric chloride solution; the formation of a blackish precipitate indicated the presence of tannins. Saponins were identified through the foam test, in which 1 mL of extract was dissolved in 5 mL of distilled water and shaken; the appearance of stable foam confirmed their presence. For flavonoids, 5 mg of extract was placed in a test tube, followed by the addition of a small amount of magnesium and a few drops of concentrated hydrochloric acid; a pink to scarlet color appearing after a few minutes indicated the presence of flavonoids.

To detect steroids, 5 mg of the herbal extract was mixed with 2 mL of chloroform, then a few drops of concentrated sulfuric acid and acetic acid were added. The formation of a greenish hue confirmed the presence of steroids. For triterpenoids, the crude extract was dissolved in 2 mL of chloroform and evaporated to dryness, after which 2 mL of concentrated sulfuric acid was added, and the mixture was heated for approximately 2 minutes. The emergence of a grayish color confirmed the presence of triterpenoids.

Alkaloids were detected using several methods. In Mayer's test, 5 mg of extract was treated with 1% hydrochloric acid and gently heated; the formation of a creamy white or yellow precipitate indicated the presence of alkaloids, due to the reaction with potassium mercuric iodide in Mayer's reagent. Wagner's test involved adding 0.5 mL of Wagner's reagent to 5 mg of extract and shaking the mixture; a reddish-brown precipitate indicated the presence of alkaloids through the formation of an insoluble iodine complex. In Dragendorff's test, one drop of Dragendorff's reagent was added to 5 mg of extract, and the appearance of an orange-red color confirmed the presence of alkaloids. This reagent, prepared using bismuth nitrate, nitric acid, iodine, and water, produces a distinctive color in the presence of alkaloids.[22,23]

Microbial strains

Two widely used *M. tuberculosis* laboratory reference strains were utilized: H37Ra (a for avirulent) and H37Rv (v for virulent). The H37Rv strain was used for in vitro laboratory-based virulence for drug sensitivity testing.[24] *Mycobacterium tuberculosis* samples, H37Rv were from Central Java Health Laboratory Center and inoculated in Lowenstein-Jensen (LJ) media.

Media preparation and herbs susceptibility test

Mycobacterium tuberculosis is an aerobic, slow-growing bacterium that typically becomes visible within 2–3 weeks, with an incubation period of up to 8 weeks at 37°C. The primary culture method employed was the Lowenstein-Jensen (LJ) medium, an egg-based medium.[25] The bacterial inoculum was prepared from solid cultures using LJ slants, where bacterial suspensions were vortexed for several minutes and then left to settle for 20 minutes to allow larger particles to precipitate.[26]

The inoculated LJ medium in McCartney bottle tubes was incubated at 37°C for up to 8 weeks and monitored every 2 weeks for macroscopic growth by trained personnel from the Tuberculosis Laboratory Team at the Central Java Regional Laboratory. Cultures that showed no visible growth after 8 weeks of incubation were considered sensitive. Any discoloration of the LJ medium or the presence of colonies of non-acid-fast bacteria was interpreted as contamination. Rifampicin was used as a positive control, while drug-free media inoculated with bacterial suspensions served as a negative control.

Antimicrobial susceptibility testing was conducted using the agar dilution method, and each experiment was performed in duplicate. This method involves incorporating varying concentrations of the antimicrobial agent into a non-selective agar medium before it solidifies. A standardized bacterial inoculum is then applied to the surface of the agar. After incubation, the plates are visually examined to assess bacterial growth at the inoculated sites. The minimum concentration of the herbal extract that inhibited visible bacterial growth was determined as the minimum inhibitory concentration (MIC). The diameter of the zone of inhibition was used to classify bacterial susceptibility into three categories: susceptible, intermediate, or resistant.[27] A strain was considered susceptible (S) when inhibited in vitro by a concentration of the herbal extract associated with a high probability of therapeutic success. It was classified as intermediate (I) when the inhibitory concentration was associated with an uncertain therapeutic outcome, and resistant (R) when the concentration required for inhibition was associated with a high risk of therapeutic failure.[28]

Ethical statement

The study was conducted at TB Laboratory in Central Java Regional Laboratory, where air contaminants standards were maintained. All procedures were performed in a Biosafety Cabinet with appropriate personal protective equipment, including respirators and gloves, to minimize the risk of airborne exposure to *M. tuberculosis*.

RESULTS

The antimicrobial activity of five herbs extract was assayed in vitro against *Mycobacterium tuberculosis* strain H37Rv. A total of 20 cultures were inoculated on LJ medium. Further examination of 12 of 20 cultures showed *Mycobacteria* grew. Among the five selected plants, the ethanol extract of *Nicotiana* spp., L and *Annona muricata* L, was found to be the most effective against *M. tuberculosis.*

Herbs are considered highly perishable due to their high moisture content. They are processed by drying to reduce moisture, which helps inhibit chemical alterations. In this study, herbs were evaluated for moisture content. *Centella asiatica* and *Annona muricata* had high moisture content, while garlic produced the highest extract yield. The plants, their respective parts used, moisture content, and extract yield are detailed in Table 1. Fresh plant material was rinsed under running tap water, allowed to air dry, ground into a fine powder, and stored in airtight containers.

Table 1. Comparison of medicinal plants description

Common name	Scientific name	Family	Part used	Moisture content (%)	Maceration (ml)	Extract (gr)
Noni	*Morinda citrifolia* L.	Rubiaceae	Fruit	8.9	2,066	14.7
Tobacco	*Nicotiana* spp., L	Solanaceae	Leaf	5.5	1,955	23.3
Pegaga	*Centella asiatica* L	Apiaceae	Leaf	9.4	1,955	22.8
Garlic	*Allium sativum* L	Amaryllidaceae	Bulb/Cloves	4.4	1,286	39.2
Soursop	*Annona muricata* L	Annonaceae	Leaf	9.4	1,975	19.5

Mycobacterium colonies exhibited a rough yellow-colored morphology on LJ medium. Growth reached its peak within 6-8 weeks. Colony morphology observations, including texture and pigmentation, were documented.

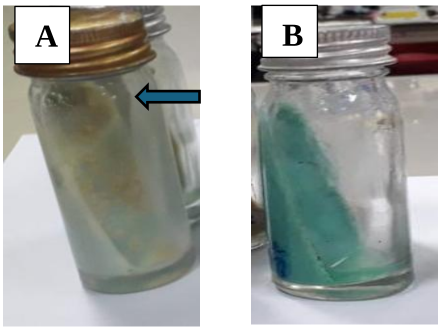

Figure 1. A comparison between positive and negative results on Lowenstein-Jensen media shows that yellow colonies of *Mycobacterium tuberculosis*, indicating positive growth, are clearly visible in image A (blue arrow), while no such colonies are present in image B. Comparison between positive and negative results on Lowenstein-Jensen media. Yellow colonies of *Mycobacterium tuberculosis* representing positive growth are clearly seen in A (blue arrow) which are absent in B.

The significant antibacterial activity of the herbal extracts was comparable to the standard antimicrobic Rifampicin (100 µg/disc) (Table 2). Tobacco and soursop exhibited good antibacterial activitiy against *M. tuberculosis* in all concentrations, whereas the remaining extracts were ineffective. Yellow colonies of *Mycobacterium tuberculosis* in Figure 2 indicate positive growth in *Morinda citrifolia* L, *Centella asiatica* L and *allium sativum* L.

Table 2. Antimicrobial activity of different concentrations from five herbs extract

Herbs	Lowensetein Jensen proportion method Inhibition rate (100%)				
	25% plant extracts	50% plant extracts	75% plant extracts	100% plant extracts	Rifampicin 100 µg/disc
Noni	0	0	0	0	100.0
Tobacco	100.0	100.0	100.0	100.0	100.0
Pegaga	0	0	0	0	100.0
Garlic	0	0	0	0	100.0
Soursop	100.0	100.0	100.0	100.0	100.0

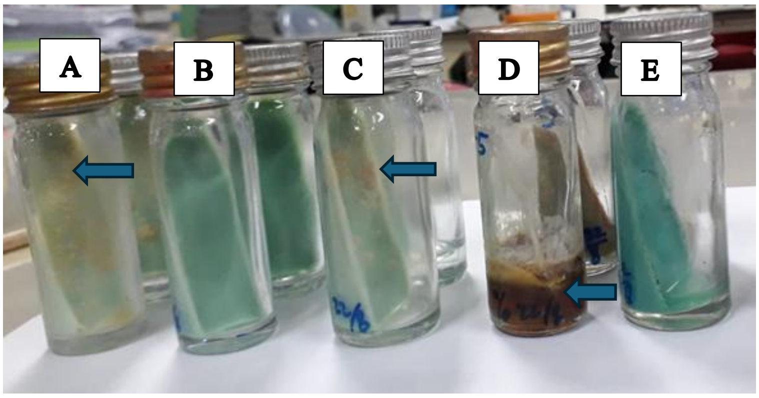

Figure 2. Comparison between antimicrobial results on Lowenstein-Jensen media. (A) *Morinda citrifolia* L. (B) *Nicotiana* spp., L (C) *Centella asiatica* L (D) *Allium sativum* L (E) *Annona muricata* L. Yellow colonies of *Mycobacterium tuberculosis* representing positive growth (blue arrows)

Tabel 3. Phytochemical screening of herb extract of the studied plants

Herbs	Tannins	Saponins	Flavonoids	Steroids	Phenolic	Triterpenoid	Alkaloids		
							MT	WT	DT
Noni	+	+	-	-	-	+	+	+	+
Tobacco	+	+	+	-	-	-	-	-	+
Pegaga	+	-	+	-	-	-	+	-	+
Garlic	+	-	-	-	-	+	-	-	+
Soursop	+	-	+	+	+	-	-	+	+

+: detected; -: not detectable; MT: Mayor's test; WT: Wagner's test; DT: Dragendorff's Test

According to the qualitative phytochemical screening, garlic sources various biologically active phytochemicals, including tannins, triterpenoids, and alkaloids. This phytochemical screening of tobacco leaf extract indicates the presence of bioactive phytochemicals tannin, saponin, flavonoid, and alkaloid.

DISCUSSION

This study investigated the in vitro antimicrobial activity of herbal extracts from five plant species—*Morinda citrifolia L., Nicotiana spp. L., Centella asiatica L., Allium sativum L., and Annona muricata L.*—against *Mycobacterium tuberculosis*. Indonesia is known for its rich plant biodiversity, offering a vast reservoir of potential medicinal resources. In light of the growing threat of antibiotic resistance, evaluating the antibacterial properties of herbal medicines is critical for identifying new therapeutic alternatives. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis has significantly complicated treatment approaches. The World Health Organization (WHO) has set ambitious targets to reduce TB mortality by 95% and incidence by 90% by the year 2035.[29] The discovery and development of new drugs are essential not only for enhancing the efficacy of TB treatment but also for improving safety profiles and mitigating both infection and adverse side effects. Common side effects of anti-TB drugs include gastrointestinal disturbances, hepatotoxicity, ototoxicity, nephrotoxicity, skin rashes, fever, peripheral neuritis, and, in rare cases, psychotic episodes.[30]

In this study, antibacterial activity was evaluated using five herbal extracts against *M. tuberculosis*. The highest activity was observed in the extracts of tobacco and soursop herbs revealed significant antituberculosis activity. Previous studies reported similar findings.[31,32] However, the antimicrobial activity of noni, pegaga and Garlic extract was not observed in our study. Some plants exhibiting anti-mycobacterial activity against multidrug-resistant tuberculosis (MDR-TB) were previously reported between 2011 and 2015 in other countries, with pegaga and garlic being mention as effective.[33] However, in this study, both plants did not show antibacterial activity, indicating that further experiments are needed.

The antimicrobial assessments of noni in this study showed resistance against *M. tuberculosis*. Previous research suggests that noni is widely used in herbal remedies due to its effectiveness in inhibiting bacterial growth.[17,19] Various parts of plants could be used for extraction of antibacterial compounds.[34] Extracts of different parts of this plant; root, stem, fruits, and leaves have been traditionally used in many countries for the treatment of numerous diseases as an antibacterial, antitumor, antihelminthic, analgesic, anti-inflammatory, and immunostimulant.[19] Noni leaves contain several chemical compounds such as terpenoids, flavonoids, saponins, and anthraquinone which act as antibacterials.[35] Our result appears to contradict previous study conducted by Saludes et al that revealed crude ethanolic extract from noni leaves showed 89% inhibition against *M. tuberculosis* H37Rv strain at 100μg/mL.[36] Another study also reported that leaves from noni fruit have been found killed *M. tuberculosis* bacteria at 40mg/ml.[37] In this study we used noni fruit while previous study used leaves. Our findings show differences in sample could be factors responsible for the results.

The tobacco extract in this study demonstrated potential antimicrobial activity against *M. tuberculosis.* This contrasts with previous studies that used isolated tuberculosis patient sputum and the control strain H37Rv, which showed resistance to both water and ethanolic extracts of Nicotiana tabacum.[38] Tobacco is an herbaceous perennial plant known to contain ingredients that are antibacterial, antifungal, and antihelmintics.[39] *Nicotiana tabacum* L possessed many pharmacological effects including antioxidant, antimicrobial, antiparasitic, analgesic, antidiabetic, antifertility, and neuropharmacological effects.[38] Extract of tobacco has been shown to effectively inhibit various Gram-positive, Gram-negative bacteria, and acid-fast.[40] Tobacco leaves have antibacterial properties because it contains important compounds such as diterpenes, steroids, flavonoids, tannins, coumarins, phenols and saponins. Flavonoids are known to inhibit inflammatory reactions, useful as antimicrobials and antioxidants.[41] Saponins are recognized for their wide range of pharmacological activities, including antimicrobial properties.[42]

In this study, the antimicrobial assessment of pegaga (*Centella asiatica*) showed no inhibitory effect against *Mycobacterium tuberculosis*. *Centella asiatica* has been traditionally used in various forms—fresh, dried, and as decoctions—for its medicinal properties. Both its leaves and roots have been utilized to treat a range of ailments associated with bacterial infections.[44] Commonly known as pegaga, this herb has been used for thousands of years due to its reputed healing properties.[45] Although several recent studies have reported that *C. asiatica* extracts exhibit antimicrobial activity[46] and possess anti-inflammatory, anti-ulcer, and memory-enhancing effects[47] our findings are consistent with other research indicating that *C. asiatica* does not exhibit any significant activity against *M. tuberculosis.*[48]

Although garlic has traditionally been used for tuberculosis treatment in some countries, our study found no antimicrobial activity of garlic extract against *Mycobacterium tuberculosis*. This contrasts with previous research in which garlic extract showed inhibitory effects against various MDR and non-MDR *M. tuberculosis* isolates. Garlic is widely consumed as a culinary ingredient due to its distinct flavor and numerous reported health benefits.[11] It has been associated with therapeutic properties for the cardiovascular system, and has shown antibacterial, antiviral, antifungal, antiprotozoal, anticancer, antioxidant, and immune-modulatory effects.[49] Recognized as one of the oldest medicinal plants, garlic has been proposed as an alternative to conventional antibiotics[50,51] showing efficacy against a broad spectrum of both gram-positive and gram-negative bacteria.[52] Its key bioactive compounds, including allicin and ajoene, contribute to its antimicrobial effects—allicin, in particular, is considered a potent natural antibiotic, sometimes even more effective than penicillin and tetracycline.[53] Additionally, garlic has been shown to combat drug-resistant bacteria by producing sulfonic acid, a compound that neutralizes harmful free radicals more rapidly than any known substance.[54] Historically, garlic has been recommended for tuberculosis treatment in both Ayurvedic and Greek medicine[55] with its in vitro effectiveness against M. tuberculosis first reported in 1946.[56] The discrepancy between our findings and those of previous studies may be due to differences in garlic species or variations in the concentration of active compounds.

In this study, soursop extract demonstrated sensitivity against *Mycobacterium tuberculosis*, aligning with previous research, such as studies by Oyekunle et al. and Tariq et al..[57,58] Phytochemical analysis of soursop extract revealed the presence of tannins, flavonoids, steroids, phenolics, and alkaloids, which is consistent with findings by Agu et al.[59] Soursop (*Annona muricata L.*) has been widely used as a traditional remedy for various diseases globally.[60] In vitro studies have characterized A. muricata L. extracts as valuable antimicrobial, anti-inflammatory, anti-protozoal, anti-neoplastic, and antioxidant agents. The plant contains active compounds, including alkaloids, flavonoids, glycosides, saponins, and tannins. Acetogenins, a class of bioactive compounds found in the leaves, are particularly known for their antibiotic properties.[61] Flavonoids, in particular, are believed to inhibit bacterial cell membrane function by forming complex compounds with extracellular and dissolved proteins, leading to membrane damage. Additionally, they can interfere with oxygen usage and inhibit bacterial metabolism.[62] The leaves of *A. muricata* have been the focus of numerous studies due to their significant medicinal properties, with earlier research confirming their potent antibacterial effects.[63]

In recent years, plant metabolites (phytochemicals) have gained significant attention as potential sources of medicinal agents.[64] The antibacterial properties of herbs are primarily attributed to their bioactive chemical compounds, such as flavonoids, alkaloids, sterols, terpenoids, phenolic acids, tannins, and saponins.[11] In this study, the extracts of various plants were analyzed for their phytochemical content. Noni contained tannins, saponins, triterpenoids, and alkaloids; tobacco contained tannins, saponins, flavonoids, and alkaloids; *Centella asiatica* contained tannins, flavonoids, and alkaloids; garlic contained tannins, triterpenoids, and alkaloids; and soursop contained tannins, flavonoids, steroids, phenolics, and alkaloids. The antibacterial effects of these herbs are likely due to the bioactive compounds present in the extracts, which act through various mechanisms to exert antibacterial activity. Flavonoids, in particular, are known for their antibacterial properties, possibly because of their ability to form complexes with extracellular proteins, soluble proteins, and bacterial cells.[65] Differences in antibacterial activities among the extracts may result from variations in chemical composition, the source of the herbs, and the mechanisms by which the bioactive components exert their effects. Antibacterial activity depends not only on the secondary metabolites in the plant extracts but also on their interaction with other compounds present in the plant.[66]

The agar dilution test used in this study has certain limitations. It is time-consuming, as it involves preparing multiple agar plates with different antimicrobial agents. Manual preparation of serial dilutions can be tedious. Additionally, agar plates have a limited shelf life and prone to chemical degradation, requiring their used within a week of preparation. The agar dilution method is also limited to testing only one antimicrobial agent at a time. Hydrophobic extracts may separate from the agar, and the use of emulsifiers and solvents may affect result accuracy. Furthermore, the results obtained from these methods do not indicate the antimicrobial agent's mode of action and may not reliably predict in vivo efficacy. Despite these drawbacks, the agar dilution method remains a valuable and widely utilized technique for antimicrobial evaluation, and is often recommended as a standardized approach for antimicrobial susceptibility testing.

CONCLUSION

This study provides a preliminary basis for selecting candidate herbs as potential anti-TB drugs. Herbs offer promise for developing alternate medicines with fewer side effects compared to synthetic antimicrobials. Further investigations are necessary to determine the efficacy. The in vitro antimicrobial activity of these herbs serves as a foundation for further phytochemical studies. Phytochemical screening is essential to assess the qualitative chemical composition of crude extracts using commonly employed precipitation methods.

CONFLICT OF INTEREST

There are no conflicts of interest.

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DATA AVALABILITY

The authors confirm that the data supporting the findings of this study are available within the article.

SUPPLEMENTAL DATA

No additional supplemental data are provided for this study. All relevant data supporting the findings of this research are included within the main article.

AUTHOR CONTRIBUTIONS

FS conceived and organized the review; FS and AN contributed to the writing and editing of the manuscript. All listed authors have made substantial, direct, and intellectual contribution to the work and have approved it for publication.

DECLARATION OF USING AI IN THE WRITING PROCESS

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

LIST OF ABBREVIATIONS

TB: Tuberculosis; LTBI: Latent TB infection; MDR-TB: multidrug-resistant TB; LJ: Lowenstein-Jensen; S: susceptible; I: intermediate; R: resistant.

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