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Antibacterial activity of Nepalese medicinal plants against different bacterial isolates

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Abstract

Antimicrobial resistance becomes a widespread issue. With this, more studies concentrating on medicinal plants are being conducted in an effort to uncover their full therapeutic potential. This study was conducted to evaluate the antibacterial activities of eight medicinal plants *(Azadirachta indica, Mentha spicata, Terminalia chebula, Terminalia bellirica, Acorus calamus, Tinospora sinensis, Ocimum tenuiflorum, Aloe vera)* in the Med-micro Research Laboratory, Babarmahal from January to July 2022. These plants were collected and using the maceration method, their extracts were made separately in distilled water, 95% ethanol, and 1% chloroform. The plant extracts were prepared using Dimethyl sulfoxide (DMSO) in two different concentrations (10% and 1%). The agar well diffusion method was used to measure the antibacterial activity of a total of 48 extracts against four bacterial isolates, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*. In contrast to gram negative bacteria, the majority of plant extracts had significant antibacterial activity against *Staphylococcus aureus*, a gram positive bacterium. The ethanolic extract of *Terminalia chebula* at a 10% concentration exhibited the largest zone of inhibition (26 mm) against *Staphylococcus aureus*. The most potent extracts were found to be those of ethanolic extract of *Terminalia chebula* and *Terminalia bellirica*, followed by those of *Acorus calamus* and *Ocimum tenuiflorum*. Our results provide additional evidence that these plants hold potential as a natural antibacterial agent, hence can be used in treatment of various infectious diseases.

Keywords: Medicinal plants, Antibacterial activity, Agar well diffusion method, Gram positive bacteria, Gram negative bacteria

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Introduction

In recent years, bacterial infections have emerged as one of the major global health threats, exacerbated by the widespread drug resistance. As such, focus has been shifted towards finding suitable alternatives to available antibiotics. Plants are a natural source of many potent and powerful drugs. Different parts of herbal plants are used as household remedies to treat many health issues.

In Nepal, the use of plants for medicinal purposes dates back to the Vedic period, between 4500 BC and 1600 BC. Ayurveda, the ancient science of life, continues to be the primary source of medical knowledge and practices in Nepal and much of South Asia. [1]. Out of a total population of approximately 29 million, nearly 80% of people reside in the rural areas of the country with very little access to government health care facilities. Thus, people mostly depend on local herbs for several health problems [2]. The antimicrobial properties of medicinal plants are attributed to several bioactive phytochemicals present such as alkaloids, tannins, terpenoids, saponins, polyphenols, etc [3]. These phytochemicals found in herbal plants have potential to kill or inhibit the pathogenic microorganisms and cure a variety of ailments including respiratory illnesses, diarrhea, and many more. Ogunmefun (2018) [4] found that medicinal plants contain various phytochemicals like saponins, which lower cholesterol; alkaloids act as stimulants; tannins act as antibiotics; anthraquinones are laxatives and dyes; glycosides act as cardiovascular drugs; phenols and flavonoid is antioxidants. An estimated 1,600 to 1,900 plant species are widely utilized in traditional medicine in Nepal [5]. Different parts of medicinal plants are used for extract as raw drugs. The use of plant extract for medicinal treatment became popular when people became aware of the limited life span of antibiotics and associated side effects. The misuse of antibiotics is one of the contributing factors in antimicrobial resistance in the modern world [6].

People always prefer the effectiveness of medicine when it comes to their illness and sometimes commercial medicines fail to provide it. Even though there are many pharmaceutical drugs to treat bacterial infections, the resistance shown by bacterial species against the drugs has increased, leading to hindrance to treat such infections.

Table 1. Medicinal plants and its constituents

Family	Plant Name	Parts Used	Active Compounds	Medicinal Uses
Araceae	*Acorus calamus* (Bojho)	Stem, Rhizomes(roots)	ß-asarone α–asarone Camphene Quinine,	Cough, sore throat Respiratory tract infections, skin diseases.
Menispermaceae	*Tinospora sinensis* (Gurjo)	Stem, Leaves, Root	Tinosporin, Xanos Boric AcidCholine, Isoclumbin	Antipyretic, Chronic Troubles of Respiratory and digestive systems urinary troubles
Lamiaceae	*Mentha spicata* (Mint)	Leaves	Kaempferol and Quercetin, Rhamnocitrin, Hesperidin	Antiseptic, treat indigestion problems, skin infections like acne headache
Meliaceae	*Azadirachta indica* (Neem)	Leaves Stem, Bark	Azadirachtin, Nimbin, Gedunin, Stearamide, Vilasinin	high antioxidant activities Malaria, insecticidal, externally applied on wounds, sores, blisters and skin diseases
Asphodelaceae	*Aloevera* (Aloe vera)	Leaves Stem	Aloin, Saponin, Anthraquinones, Aloe-emodin, Barbalion	Inflammations Cooling Effect, Skin infections, Laxatives. Antifungal, Hair Fall solution and cosmetic products.
Combretaceae	*Terminalia chebula* (Harro)	Stem Fruit	Chebulagic Acid Gallic Acid, Ellagic acid, Terchubin	Teeth and Gum problems , Tonics and Ayurvedic powder 'Triphala'.
Combretaceae	*Terminalia bellirica* (Barro)	Fruit Stem	Gallic acid, Punigluconin, Phyllembin, Ellagitannins	Cough and hoarseness and also used as laxatives and tonics, Digestive issues
Lamiaceae	*Ocimum tenuiflorum* (Tulsi)	Leaves Stem	β-sitosterol, Eugenol, Elixin, Apigenin, Luteolin, Ocimene, Transanethole	As tonics and drops. Cough fevers, Sore Throat and cold, Asthma and influenza.

Source: [7, 8, 9, 10, 11, 12, 13]

In recent years, plant products have been sought as a source of naturally derived antimicrobials and an alternative to available pharmaceuticals. The effectiveness of plant extracts for antimicrobial therapy has been observed to yield promising results since ancient times. The main reasons for extensive use of medicinal plants are because they are reliable, cheaper and less toxic compared to their chemically synthesized counterparts and have easy access to people all around.

The future of medicinal plants is promising, as a large number of medicinal plants have been discovered, but their phytochemical composition has yet to be explored. It has become a necessity to fulfill the demand of alternative medicine. Since herbal plants are accessible to local communities, it could help improve the socio economic status of people as well as the nation. Therefore, the present study aims to assess the antibacterial properties of Nepalese medicinal plants against bacterial isolates.

Materials and methods

Plant sample collection

Eight plant species, namely *Azadirachta indica* (Neem), *Mentha spicata* (Mint), *Terminalia chebula* (Harro), *Terminalia bellirica* (Barro), *Acorus calamus* (Bhojo), *Tinospora sinensis* (Gurjo), *Ocimum tenuiflorum* (Tulsi) and Aloe v*era* (Aloe) were collected aseptically from different parts of Kathmandu valley and further processed in the Med-micro Research Laboratory, from January to July 2022. Fresh leaves of *Aloe vera, Ocimum tenuiflorum, Mentha spicata* as well as *Azadirachta indica*, stem of *Acorus calamus, Tinospora sinensis*, and fruits of *Terminalia bellirica, Terminalia chebula* were selected for their medicinal properties as per the literature. In sterile condition, each plant part was washed, shade-dried and powdered for extract preparation.

Preparation of plant extracts

The plant extracts were prepared using a maceration method [14]. 10g of each obtained plant powder was dissolved in distilled water, 95% ethanol and 1% chloroform each, in different conical flasks in a ratio of 1:10. This mixture was allowed to soak for five days with periodic agitation in these tightly sealed flasks. Afterwards, the mixture was filtered and the resulting extract (filtrate) was concentrated in a hot air oven set at 45°C. The dried extract was scraped and 0.1g of this extract was suspended into 1 mL of 1% Dimethyl Sulfoxide (DMSO) to obtain 10% concentration and then 0.1 mL of 10 % extract was dissolved in 0.9 mL of 1 % DMSO to achieve 1% concentration.

Drying of *Mentha* leaves.

Plant Extract preparation (Soaking).

Confirmation tests for bacterial isolates

The bacteria used for this study included *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were obtained from Med-micro Research Laboratory. The bacterial isolates were confirmed by Gram staining, Catalase test, Oxidase test, Oxidative Fermentative test, Coagulase test and Biochemical tests (Indole test, Methyl red test, Voges Proskauer Test, Citrate utilization test, TSIA test and Urease test).

Preparation of test inoculum

Each bacterial isolate was sub-cultured into nutrient broth at 37°C for 2-3 hours. The test suspension was standardized to match 0.5 McFarland turbidity standard which corresponds to concentration of 1.5×10^8 cells/mL [15].

Agar well diffusion assay

Agar well diffusion method was used to determine the antibacterial activity of plant extracts. The test bacterial inoculum was carpet cultured onto Mueller Hinton Agar with a sterile cotton swab moistened with the bacterial suspension. Five wells of 6 mm were made with sterile cork borer. Then, 45 uL of 10% and 1% concentration of each of eight plant extracts were loaded onto the 4 wells and 1% DMSO was added in the central well as negative control. The zone of inhibition was measured following an overnight incubation [16]. The test was repeated to ensure the reliability of the results.

Quality Control

The reagents and media were freshly prepared and were supervised for expiry date and proper storage. The bacterial isolates were subcultured on a regular basis on a nutrient agar plate. The plant extracts were stored in airtight vessels. The cork borer was sterilized by using an autoclave. Used media and materials were sterilized by autoclaving at 121°C at 15 lbs. pressure for 15 minutes before disposal.

Results

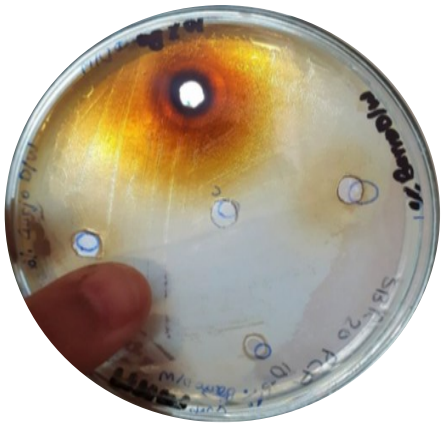

Figure 1. ZOI observed against *S. aureus* for Aqueous extracts (10% and 1%) of *Tinospora sinensis* and *Terminalia bellirica*.

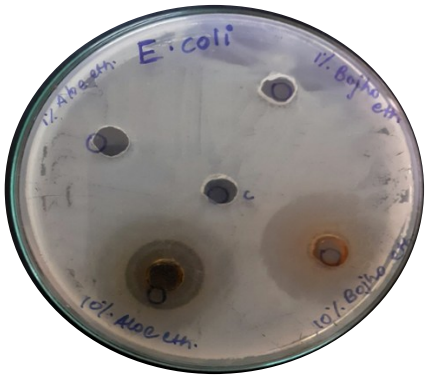

Figure 2. Zone of inhibition as observed against *Escherichia coli* for Ethanolic extracts (10% and 1%) of *Acorus calamus* and *Aloe vera*.

In this study, in vitro antibacterial activity of eight Nepalese medicinal plants were assessed against four bacterial isolates. All the plant extracts yielded most effective results against *S. aureus*. The maximum zone of inhibition (26 mm) in 10% concentration of ethanolic extract of *Terminalia chebula* was shown against *S aureus* followed by 10% ethanolic extract of *Terminalia bellerica* i.e. 22 mm. Similarly, 10% concentration ethanolic extract of *Acorus Calamus* (bojho) showed a maximum zone of inhibition i.e. 16 mm (**Table 2**).

Table 2. ZOI shown against *Staphylococcus aureus* by medicinal plant extracts.

S.N.	Sample used	ZOI in 10% concentration (in mm)			ZOI in 1% concentration (in mm)		
		Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform
1	*Azadirachta indica* (Neem)	12	12	17.5	8	8	13
2	*Mentha spicata* (Mint)	14	13	17	0	8.5	0
3	*Terminalia chebula (* Harro)	24	26	17	10	15	9
4	*Terminalia bellirica* (Barro)	16	22	15	7.5	11	8
5	*Acorus calamus* (Bojho)	14	16	13	0	6.5	9
6	*Tinospora sinensis* (Gurjo)	10	0	18	9.5	0	9
7	*Ocimum tenuiflorum* (Tulsi)	11	17	15	7	7	7
8	*Aloe vera* (Aloe)	8	11	22	7	7	13

Table 3. ZOI shown against *Escherichia coli* by medicinal plant extracts.

S.N.	Sample used	ZOI in 10% concentration (in mm)			ZOI in 1% concentration (in mm)		
		aqueous	ethanol	chloroform	aqueous	ethanol	chloroform
1	*Azadirachta indica* (Neem)	0	0	0	0	0	0
2	*Mentha spicata* (Mint)	0	0	0	0	0	0
3	*Terminalia chebula (* Harro)	15	21	12	0	0	0
4	*Terminalia bellirica* (Barro)	16	11	15	8	16	10
5	*Acorus calamus* (Bojho)	8	25	11	0	0	0
6	*Tinospora sinensis* (Gurjo)	0	0	0	0	0	0
7	*Ocimum tenuiflorum* (Tulsi)	7	7	0	0	0	0
8	*Aloe vera* (Aloe)	0	14	14	0	0	0

Table 4: ZOI shown against *Klebsiella pneumoniae* by medicinal plant extracts.

S.N.	Sample used	ZOI in 10% concentration (in mm)			ZOI in 1% concentration (in mm)		
		aqueous	ethanol	chloroform	aqueous	ethanol	chloroform
1	*Azadirachta indica* (Neem)	0	13	17.5	0	0	0
2	*Mentha spicata* (Mint)	17	15.5	16	14	0	14
3	*Terminalia chebula* (Harro)	21.75	24	21.5	19	22	15
4	*Terminalia bellirica* (Barro)	18	14	17	0	12	9
5	*Acorus calamus* (Bojho)	17.75	0	12.75	13	0	11
6	*Tinospora sinensis* (Gurjo)	0	0	0	0	0	0
7	*Ocimum tenuiflorum* (Tulsi)	10	15	16	18	16.5	0
8	*Aloe vera* (Aloe)	13	0	19	0	0	10.75

Table 5: ZOI shown against *Pseudomonas aeruginosa* by medicinal plant extracts.

S.N.	Sample used	ZOI in 10% concentration (in mm)			ZOI in 1% concentration (in mm)		
		aqueous	ethanol	chloroform	aqueous	ethanol	chloroform
1	*Azadirachta indica* (Neem)	0	0	0	0	0	0
2	*Mentha spicata* (Mint)	0	0	0	0	0	0
3	*Terminalia chebula (* Harro)	24	22	26	13	18	16
4	*Terminalia bellirica* (Barro)	0	23	15	0	16	11.5
5	*Acorus calamus* (Bojho)	16.75	15	12	10	0	8
6	*Tinospora sinensis* (Gurjo)	0	7	0	0	0	0
7	*Ocimum tenuiflorum* (Tulsi)	14.5	21	8	11.5	20	0
8	*Aloe vera* (Aloe)	9.75	0	9.75	0	0	0

The ethanolic extracts of most plants were effective against *S. aureus* followed by aqueous extracts of 10 % concentration. For 1% concentration, chloroform extract of *Azadirachta indica*(Neem) was most effective with ZOI of 13 mm followed by 1% ethanolic extract of *Terminalia bellirica*(barro) showing ZOI of 11 mm.

Against *Escherichia coli*, the maximum ZOI was shown by 10% ethanolic extract of *Acorus calamus* i.e. 25 mm followed by 10% ethanolic extract of *Terminalia chebula* (harro) showing ZOI of 21 mm (**Table 3**). Both chloroform and ethanolic extracts of Aloe vera at 10 % concentration yielded ZOI of 14 mm against *E coli*. For 1% ethanolic extract of *Terminalia bellirica* showed maximum ZOI of 16 mm as other extracts were ineffective in the same concentration. The extracts of *Mentha spicata, Azadirachta indica* and *Tinospora sinensis*(gurjo) were not effective against *Escherichia coli* since no zone of inhibition was observed in both 10 % and 1% concentration.

Against *K. pneumoniae,* the maximum ZOI was shown by 10% and 1% ethanolic extract of *Terminalia chebula* (Harro) i.e. 24 mm and 22 mm respectively. It was followed by 10% aqueous extract of *Terminalia chebula* yielding an ZOI of 21.75 mm. For chloroform extract, maximum ZOI of 21.5 mm was shown by 10% extract of *Terminalia chebula* followed by ZOI of 19 mm shown by 10% *Aloe vera* extract. *Tinospora sinensis* was ineffective against *K. pneumoniae* showing no zone of inhibition (**Table 4**).

For *Pseudomonas aeruginosa*, the maximum ZOI (26 mm) was shown by 10% chloroform extract of *Terminalla chebula* followed by its 10 % aqueous extract i.e. 24 mm. 10% ethanolic extract of *Terminalia bellirica* showed ZOI of 23 mm. 10% ethanolic extract of *Acorus calamus* (Bojho) was also effective against it, showing 16.5 mm ZOI. For 1% concentration, the ethanolic leaf extract of *Ocimum tenuiflorum* was found most effective showing the ZOI of 20 mm followed by *Terminalia chebula* with ZOI of 18 mm. Leaf extract of *Mentha spicata* and *Azadirachta indica* were not effective against *P. aeruginosa* as no ZOI was observed in all concentrations of three solvents (**Table 5**).

Discussion

Among all the extracts assessed, the extracts of *Terminalia chebula* (Harro) and *Terminalia bellirica* (Barro) were found to be most potent showing maximum activity against the test bacteria at both 10% and 1% concentration of all solvents. It is followed by 10% chloroform extract of *Azadirachta indica* (Neem). 1% ethanolic extract of *Ocimum tenuiflorum* (Tulsi) was also effective against the test organisms. Other plant extracts showed moderate antibacterial activities. *Tinospora sinensis* (Gurjo) was found to be least effective showing less inhibitory effect in both 10% and 1% concentration. This could potentially be due to the choice of solvent utilized during the extraction process. Additionally, the loss of active phytochemicals inherent in the plant during the maceration process (including drying and extraction) may also contribute to its reduced effectiveness.

The plant extracts demonstrated more effective results against gram-positive bacteria as compared to gram-negative bacteria. In a study conducted by Joshi, et al. (2010) [17], on selected medicinal plants viz. *Ocimum sanctum* (Tulsi), *Cinammomum zelanicum* (Dalchini), *Origanum majorana (*Ram tulsi), *and Zanthoxylum armatum* (Timur*)* against the ten pathogenic bacterial strains, these plant extracts were more effective against gram-positive bacteria than gram-negative bacteria. which corresponds with our result. In a similar study done by Pangeni, et al., (2021) [18], twenty-five ethno medicinal plants were tested against four bacteria. Out of 50 samples, 38 plant extracts demonstrated a stronger effect against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), while 31 plant extracts were effective against gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The difference in effectiveness toward plant extracts demonstrated by two groups of bacteria may be due to the structural variations between the two groups of bacteria, such as the composition of their cell walls, presence of resistance genes and other factors. Gram negative bacteria have outer membranes enriched with lipopolysaccharide (LPS) and the presence of periplasmic space accounts for such relative resistance against many environmental substances including antibiotics [19].

All of the plant extracts used in this study contain one or more secondary metabolites (**Table 1**). Maceration method is convenient and very effective for thermolabile plant material and don't degrade metabolites during the extraction process. [14,20] In this study, solvents with different polarities were used to prepare various plant extracts, each influencing the extraction of distinct classes of bioactive compounds. Water and alcohol, both of which are highly polar solvents, were used to extract polar secondary metabolites from the plant parts, including flavonoids, phenolic compounds, and alkaloids, which are known for their antibacterial properties.[14,20,21] Chloroform, being a nonpolar solvent, was utilized for the extraction of nonpolar bioactive compounds such as terpenoids and lipophilic flavonoids, which also contribute to the antibacterial activity but may do so through different mechanisms, such as membrane disruption or inhibition of metabolic

processes [14,20,21]. The observed differences in antibacterial activity can, therefore, be attributed to the varying solubility and bioactivity of these compounds, depending on the solvent used for extraction.

In a study by Reygaurt, WC. (2018), it was highlighted that microorganisms use various resistance strategies, including efflux pumps, enzyme inactivation, target modification, and changes to the microenvironment, to make antibiotics ineffective [22]. These resistance mechanisms present major obstacles for the pharmaceutical industry in the development of new drugs. The process of developing new antibiotics is time-consuming and extremely costly. Secondary metabolites from plants have demonstrated potential as treatments for infections and various health disorders. The use of plants has increased and has shown better results than synthetic drugs as it doesn't have any side effects and as compared to chemically derived drugs [23]. If explored further, these plant extracts can be used in pharmaceutical industries for production of effective drugs against bacterial species especially in the developing countries where antimicrobial resistance is more prevalent and rising each year.

Conclusions

In this study, we assessed the in-vitro antibacterial activity of eight herbal plants native to Nepal. The extracts of *Terminalia chebula* and *Terminalia bellirica* were found to be most potent, showing a broad-spectrum activity against the test bacteria at both 10% and 1% concentrations. *Tinospora sinensis* extracts were found to be least effective. Overall, all the plant extracts yielded most effective results against *Staphylococcus aureus*. The extracts of these said plants can be utilized as a potential source in pharmaceutical industries to treat various bacterial infections.

Future studies can focus on isolating and identifying the active compounds responsible for antibacterial activity, as well as conducting in-vivo testing to assess their safety, efficacy, and optimal concentration. Relatively lower antibacterial activity found in this study, can also be explored in combination with more potent extracts for synergistic effects.

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Declaration of Interest

The authors report that there is no conflict of interest.

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