Evaluation of Phytochemical, Antioxidant and Antibacterial Activities of Selected Medicinal Plants

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Abstract

Medicinal plants are important reservoirs of bioactive compounds that need to be explored systematically. Because of their chemical diversity, natural products provide limitless possibilities for new drug discovery. This study aimed to investigate the biochemical properties of crude extracts from fifteen Nepalese medicinal plants. The total phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant activity were evaluated through a colorimetric approach while the antibacterial activities were studied through the measurement of the zone of inhibition (ZoI) by agar well diffusion method along with minimum inhibitory concentrations (MIC) by broth dilution method. The methanolic extracts of Acacia catechu and Eupatorium adenophorum showed the highest TPC (55.21 ± 11.09 mg GAE/gm) and TFC (10.23 ± 1.07 mg QE/gm) among the studied plant extracts. Acacia catechu showed effective antioxidant properties with an IC50 value of 1.3 μg/mL, followed by extracts of Myricia esculenta, Syzygium cumini, and Mangifera indica. Morus australis exhibited antibacterial activity against Klebsiella pneumoniae (ZoI: 25mm, MIC: 0.012 mg/mL), Staphylococcus aureus ATCC 25923 (ZoI: 22 mm, MIC: 0.012 mg/mL), Pseudomonas aeruginosa (ZoI: 20 mm, MIC: 0.05 mg/mL), and methicillin-resistant Staphylococcus aureus (MRSA) (ZoI: 19 mm, MIC: 0.19 mg/mL). Morus australis extract showed a broad-spectrum antibacterial activity, followed by Eclipta prostrata, and Hypericum cordifolium. Future study is recommended to explore secondary metabolites of those medicinal plants to uncover further clinical efficacy.

Keywords: Antibacterial activity; Medicinal plants; Secondary metabolites; Minimum inhibitory concentration

Introduction

The separation and identification of physiologically active chemicals and molecules from medicinal plants has resulted in innovative treatments and pharmaceutical advances. Secondary metabolites extracted from medicinal plants have played a significant role in upholding human health against various infectious diseases since ancient times. Plant extracts or their active phytoconstituents have been used as folk medicine by 80% of the world’s population in conventional therapies [1]. It is believed that over 50% of all modern clinical drugs are of natural product origin [2].

Multidrug resistance (MDR) is characterized as an acquired non-susceptibility to at least one antimicrobial agent from three or more categories [3]. Mobile genetic elements such as interferons, plasmids, and transposons are the most common carriers of antibiotic resistance among bacteria [4]. The rapid emergence of resistance to newly introduced antimicrobial agents, suggests that even a new antimicrobial agent would not be a complete solution to the problem [5]. MDR pathogens have raised a significant problem in public health by undermining the existing antibiotic-based treatment era, resulting in an increased mortality rate in patients [6]. MDR pathogens worsen the disease severity and put the value of antibiotics at risk, affecting the global economy [7]. It is anticipated that if the race of antimicrobial resistance (AMR) keep rising, it would take the lives of nearly ten million peoples annually by 2050 [8]. Thus, a new antibacterial agent is urgently needed to treat MDR-induced infections caused by pathogens such as Enterobacteriaceae, Staphylococcus aureus, extended-
spectrum β-lactamase (ESBL) producing bacteria, among others [9].

Table 1: Description of medicinal plants used in this study.

| Medicinal plants                | Voucher specimen | Local name | Parts used  |
|---------------------------------|------------------|------------|-------------|
| Eclipta prostrata               | NCDB203          | Bhringaraj | Whole plants|
| Shorea robusta                  | NCDB212          | Saal       | Leaves      |
| Smalланthus sonchifolius        | NCDB214          | Yacon      | Leaves      |
| Hypericum cordifolium           | NCDB201          | Arelu      | Leaves      |
| Mangifera indica                | NCDB211          | Mango      | Leaves      |
| Morus australis                 | NCDB210          | Kimbu      | Barks       |
| Psidium guajava                 | NCDB206          | Guava      | Leaves      |
| Chrysanthemum indicum           | NCDB205          | Godawari   | Leaves      |
| Myrica esculenta                | NCDB208          | Kafal      | Leaves      |
| Urtica ardens                   | NCDB213          | Sisnoo     | Buds        |
| Pterocarpus marsupium           | NCDB204          | Bijayasal  | Barks       |
| Eupatorium adenophorum           | NCDB202          | Banmara    | Leaves      |
| Zingiber officinalis            | NCDB200          | Aduwa      | Leaves      |
| Accacia catechu                 | NCDB209          | Khair      | Barks       |
| Syzygium cumini                 | NCDB207          | Jamun      | Leaves      |

Acinetobacter baumannii, Pseudomonas aeruginosa, Medicinal plants produce secondary metabolites that can tackle MDR pathogens. Furthermore, medicinal plants have immunomodulatory and antioxidant activity, which result in antibacterial properties. They have a wide range of immunomodulatory effects stimulating both non-specific and specific immunity [10]. Antimicrobial and antioxidant activity is found in phytochemicals such as vitamins (A, C, E, and K), tannins, carotenoids, polyphenols, flavonoids, alkaloids, saponins, pigments, enzymes, terpenoids, and minerals [11]. Nonetheless, analgesic, antibacterial, deodorizing, febrifuge, fungicidal, antiseptic, astrigent, galactagogue, diuretic, antidepressant, insecticidal, antipyretic, and sedative properties have been recorded for volatile oils from plants (Blanco et al., 2009; Bekoe et al., 2018; Iscan et al. 2002).

However, microorganisms have continuously evolved with a wide range of metabolic mechanisms to overcome drug effects [6]. Plant-derived drugs are a superior choice over synthetic drugs because of fewer side effects and adverse effects (Bindu Jacob & Narendhirakannan R.T., 2019; Verma et al., 2018). Nepal is rich in biodiversity and geographical condition with diverse flora, and numerous species are believed to possess curative properties. However, most of these claims lack scientific validation. The plants selected for this study are being used routinely by the indigenous people as remedies against various human diseases since ancient times. Therefore, the selected plants may contain certain important bioactive compounds that could have some medicinal and antimicrobial properties and some therapeutic value based on phytochemical constituents and their secondary metabolites. Hence, the antibacterial activity of plant extracts reported here would be beneficial to identify some potent secondary metabolites as future drug candidates for the therapeutic measures of MDR-strains-induced infections in Nepal and beyond.

Materials and Methods

Bacterial isolates

Eight MDR bacterial strains: Acinetobacter spp. (628), Citrobacter freundii (377), methicillin-resistant Staphylococcus aureus (MRSA) (338), Klebsiella pneumoniae (386), Pseudomonas aeruginosa (484), Escherichia coli (2A), Morganella morganii (4331), and Xanthomonas spp. (767) were collected from the National Public Health Laboratory (NPHL), Kathmandu, and transferred aseptically to the laboratory of the Department of Biotechnology, National College for further study. All isolates were obtained from clinical specimens. Besides, ATCC strains such as E. coli 25922, S. aureus 25923, Salmonella Typhimurium 14028, and K. pneumoniae 700603 were also collected from the NPHL stored at -20°C for further studies.

Collection of plant materials

Different parts (leaves, bark, fruit, roots, and stem) were collected based on the ethnomedicinal and traditional medicinal practices from different geographical regions of Nepal as depicted in Table 1 (Collection period: January to June 2017). The plant samples were identified by National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal, and herbarium collections were deposited in the Department of Botany, National College, Khusibu, Kathmandu.

Preparation of plant extracts

The plant parts (mentioned in Table 1) were dried in the shade at room temperature, pulverized into the powders with the help of a grinding mill, and then soaked in methanol for 24 hours. Then, they were filtered, and the process was repeated three times with fresh methanol. To obtain plant extracts, the filtrates were concentrated in a rotary evaporator at 50 °C.

Determination of TPC and TFC

Using Folin-Ciocalteu reagent and a 96-well plate-based colorimetric process, The TPC was calculated (Ainsworth & Gillespie, 2007; Bhandari et al., 2021). Initially, 20 μL of plant extract was mixed with 100 μL of
Folin-Ciocalteu's reagent (1:10 v/v) and 80 µL of sodium carbonate (7.5%, w/v) in each well-containing standard and sample before incubation. Then, the sample was incubated at room temperature, and absorbance was measured at 765 nm [15]. By comparing TPC to standard gallic acid, milligrams of gallic acid equivalents per gram of extract (mg GAE/gm) were determined. Likewise, for TFC, 20 µL of plant extract was mixed with 60 µL of methanol, 5 µL of potassium acetate (1 M), 5 µL of 10% aluminum chloride, and 110 µL of distilled water, then incubated at room temperature for 30 minutes, and the absorbance was measured at 415 nm [17]. Likewise, TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/gm) by comparing to standard quercetin [17].

**Determination of antioxidant activity**

The antioxidant property was determined by discoloration assay based on the scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical (0.1 mM) (Brand-Williams et al., 1995; Aryal et al., 2021) at 517 nm using a multi-plate reader (Epoch 2, BioTek, Instruments, Inc., USA), maintaining 1 mg/mL of quercetin as a control. Crude extracts were allowed to react with DPPH free radicals for 30 minutes at room temperature. The scavenging of DPPH radical was calculated by using the following expression: (where optical density (OD) is the absorbance).

\[
\text{% Scavenging} = 100 - \frac{\text{OD of extract}}{\text{OD of control}} \times 100
\]

**Antibacterial activity**

Using sterile cotton swabs moistened with the bacterial suspension, an inoculum suspension containing 1.5 x 10^8 CFU/mL of bacteria was spread on firm Muller-Hinton Agar (MHA) plates (Balouiri et al., 2016; Marasini et al., 2015; Valgas et al., 2007). Using a sterile cork borer, wells were punched in plates (6 mm diameter) and micropipettes were used to fill the wells with a functioning suspension (50 µL) of plant extracts (50 mg/mL), as well as neomycin (20 µg/mL), amikacin (30 mcg), and nitrofurantoin (30 mcg) as positive controls and 50% DMSO as negative controls [23]. The MHA plates were incubated for 24 hours at 37°C and finally, the ZoI was determined after overnight incubation.

**Determination of MIC**

The broth dilution method was followed to determine MIC values of plant extracts as recommended by the Clinical and Laboratory Standards Institute [24]. Extracts of *E. adenophorum*, *M. australis*, *E. prostrata*, *A. catechu*, *Z. officinale*, *P. marsupium*, *S. robusta*, *M. indica*, *S. sonchifolius*, *M. esculenta*, *U. ardens*, *H. cordifolium*, *S. cumini*, *P. guajava*, and *C. indicium* showed significant antibacterial activity with larger ZoI, so they were selected for the determination of MIC value. The plant extracts were two-fold diluted to get a series of concentrations ranging from 25 mg/mL to 0.012 mg/mL in freshly prepared sterile nutrient broth. Then 20 µL of bacterial culture adjusted to 0.5 McFarland Standard was inoculated in each dilution tube and incubated at 37°C for 24 hours. The set-up included bacterial growth controls containing test tubes with media inoculated with 20 µL of bacterial inoculum only and negative controls with media and plant extract without bacterial inoculum. The MIC value was measured by choosing the lowest concentration of plant extract that inhibited the organism’s growth in the test tubes, as determined by unaided observation. The bacterial growth in the tubes containing the plant extracts was compared to the control sample without the plant extracts to establish the growth endpoints. Each assay was carried out in triplicate to confirm the results.

**Results**

The researches on medicinal plants have been carried throughout the world to explore the bioactive compounds which could be used to make a preventive or treatment approach against various health complications. The ethnopharmacological applications of plants under study were depicted in Table 2.

**Yields, TPC and TFC of plant extracts**

The percentage yield of plant extracts varied from 5.94% to 28.47% (Table 3). Extracts of *H. cordifolium* had the highest percentage yield (28.47%), followed by *A. catechu* (23.0%), *P. guajava* (21.82%), and *M. esculenta* (19.02%). Noticeably all plant extracts were found to be in semi-solid inconsistency.
Ferulic acid and Catechins possess anti-oxidative, etc. 

Table 2: Medicinal plants selected under study with their ethnopharmacological applications

| Medicinal plants         | Family         | Ethnopharmacological applications                                                                                                                                                                                                 |
|--------------------------|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| *Eclipta prostrata*      | Asteraceae     | Used as an anti-inflammatory, antivenom [25], anti-aging, hepatoprotective, anti-viral, antimicrobial agents. Bithiophenes and 5-(but-3-yn-1-2-diol)-5'-hydroxy-methyl-2,2'-bithiophene isolated from this plant used as antibacterial and antihyperglycemic [26], [27]. |
| *Shorea robusta*         | Dipterocarpaceae | Used in the treatment of ulcer, cough, itching, leprosy, and anthelmintic [28]. Antibacterial wound healing and anti-inflammatory activity due to the presence of polyphenols, flavonoids, and triterpenoids, etc. Ursolic acid extracted from this plant is responsible for showing antibacterial activity [29]. |
| *Smallanthus sonchifolius* | Asteraceae     | Leaves extract contains the compounds flucutanin and enhydrin show antibacterial activity [31]. |
| *Hypericum cordifolium*  | Hypericaceae    | Treatment of back pain and broken bones, an antidepressant [32]. Dermatological, neurological, and traumatological problems, antibacterial activity [33]. |
| *Mangifera indica*       | Anacardiaceae   | Used for gastric disorders, mouth sores, tooth pain, and dermatological disorders. [34] Treatment for diabetes, infertility, ethanolic extract of *M. indica* showed significant antibacterial activity. Methanolic extract displayed cytotoxicity against the pancreatic cancer cell line. Magniferin (5) from plant extract showed antimicrobial effect [35], [36]. |
| *Morus australis*        | Moraceae        | Treatment for fever, protect the liver, improve eyesight, strengthen joints, lower blood pressure [37]. Leaves contain 1-deoxynojirimycin known to have potential α-glucosidase inhibition activity. The piperidine alkaloid and glycoproteins from the extract of *M. australis* have been used for antidiabetic agents [38]. |
| *Psidium guajava*        | Myrtaceae       | Used for ulcers, wounds, toothache, anti-allergic effects, anti-cancer effects, and anti-hyperglycemia [39]. Used effectively in diabetes, diarrhea, dysentery, pain relief, cough, gastroenteritis, hypertension, and caries. The hypoglycemic components in *Psidium guajava* might be due to oleanolic acid, arjunolic acid, ursolic acid, and glauceric acid [40]. |
| *Chrysanthemum indicum*  | Asteraceae      | Used for hypertension, pneumonia, colitis, stomatitis, fever, neurological problems, headache [41], antipyretic property, treatment of cephalgia, vertigo, and eye inflammations [42]. |
| *Myrica esculenta*       | Myricaceae      | Used for cough, anemia, asthma, chronic dysentery, fever, sores, tumors, nasal catarrh, piles, throat complaints, ulcers, and urinary discharges[43]. Used against different disease conditions such as; antidiabetic, antiallergic, antimicrobial, anti-ulcer, anti-hypertensive, antioxidant, and higher phenolic and flavonoid compounds including myricetin, myricanol, and myricanone have anti-inflammatory properties. [44]. |
| *Urtica ardens*          | Urticaceae      | Used for diabetes, diarrhea, excessive menstrual bleeding, urinary disorders, respiratory problems, ulcers, asthma, rheumatism, high blood pressure [45]. Treatment for sprains, kidney stones, hemorrhoids, flu, fever, hepatoprotective, nephroprotective effect, etc. [46]. |
| *Pterocarpus marsupium*  | Fabaceae        | Stomachache, cholera, dysentery, urinary complaints, tongue disease, toothache, and cough are all treated. [47]. Treatment of diabetes, jaundice, and an ulcer [48]. Used for treatment of emetic, diaphoretic, stimulant, tonic, fever, colds and wounds, analgesic [49]. Used as an anti-inflammatory, blood coagulant, antimicrobial, anti-septic, and analgesic, antipyretic. Isomers of mono-cafeoylquinic acid present in *E. adenophorum* exhibit potent anti-inflammatory, anti-bacterium, and anti-obesity properties [50]. |
| *Eupatorium adenophorum*  | Asteraceae      | Treatment of diabetes, high blood pressure, cancer, stomachache, nausea, asthma, respiratory disorders [51]. Treatment for diabetes, blood pressure, stomach ache, weight loss, diarrhea, and nausea. Geraniol present in *Z. officinale* shows potential anti-inflammatory and antioxidant effects [52]. It can be used to treat colds, coughs, ulcers, boils, and skin eruptions, bleeding masses, antipyretics, and acute and chronic wound healing. [53]. The key constituents of *A. catechu* are catechin and taxifolin, which have antifungal, antiviral, antibacterial, anti-inflammatory, and antioxidant properties. [53]. |
| *Zingiber officinale*    | Zingiberaceae   | Used for diabetes mellitus, constipation, stomachache, HIV, inflammation leucorhoea, fever, strangury, and dermopathy [54], [55]. Ferulic acid and Catechins possess antioxidant properties [56]. Gallo catechins are used to treat diabetes. Quercetin isolated from *S. cumini* is used to treat diabetes and treat cytotoxicity. |
| *Acacia catechu*         | Fabaceae        | Used for diabetes mellitus, constipation, stomachache, HIV, inflammation leucorhoea, fever, strangury, and dermopathy [54], [55]. Ferulic acid and Catechins possess antioxidant properties [56]. Gallo catechins are used to treat diabetes. Quercetin isolated from *S. cumini* is used to treat diabetes and treat cytotoxicity. |
Table 3: Physical characteristics and percentage yield of the crude extracts.

| Medicinal plants       | Local Name | Dry weight of plant (gm) | Percentage yield (%) |
|------------------------|------------|--------------------------|----------------------|
| Hypericum cordifolium  | Arelu      | 40                       | 28.46                |
| Acacia catechu         | Khayr      | 50                       | 23.0                 |
| Psidium guajava        | Guava      | 50                       | 21.82                |
| Myrica esculenta       | Kafal      | 50                       | 19.02                |
| Syzygium cumini        | Jamun      | 50                       | 17.0                 |
| Mangifera indica       | Mango      | 50                       | 14.9                 |
| Chrysanthemum indicium | Godawari   | 50                       | 13.44                |
| Zingiber officinale    | Ginger     | 50                       | 12.5                 |
| Smallanthus sonchifolius | Ground apple | 50             | 11.16                |
| Pterocarpus marsupium  | Bijayasal  | 50                       | 11.02                |
| Eupoterium adenophorum | Banmara    | 50                       | 10.42                |
| Shorea robusta         | Sal        | 50                       | 9.1                  |
| Eclipta prostrata      | Bhringraj  | 70                       | 6.54                 |
| Morus australis        | Kimbu      | 34.8                     | 6.03                 |
| Urtica ardens          | Sisnoo     | 50                       | 5.94                 |

Table 4: TPC of medicinal plants.

| Medicinal plants       | TPC (mg GAE/gm) |
|------------------------|----------------|
| Acacia catechu         | 55.21 ± 11.09  |
| Urtica ardens          | 50.01 ± 5.0    |
| Mangifera indica       | 49.88 ± 19.2   |
| Psidium guajava        | 45.21 ± 2.73   |
| Shorea robusta         | 45.21 ± 4.15   |
| Eupoterium adenophorum | 37.61 ± 4.14   |
| Hypericum cordifolium  | 36.28 ± 2.37   |
| Chrysanthemum indicium | 32.95 ± 4.43   |
| Syzygium cumini        | 28.28 ± 1.85   |
| Myrica esculenta       | 23.21 ± 4.42   |
| Pterocarpus marsupium  | 22.68 ± 1.35   |
| Morus australis        | 19.75 ± 2.94   |
| Zingiber officinale    | 19.21 ± 2.0    |
| Eclipta prostrata      | 18.95 ± 1.24   |
| Smallanthus sonchifolius | 9.08 ± 1.01 |

Table 5: TFC of medicinal plants.

| Medicinal plants       | TFC (mg QE/gm) |
|------------------------|---------------|
| Eupoterium adenophorum | 10.23 ± 1.07  |
| Morus australis        | 9.10 ± 0.98   |
| Eclipta prostrata      | 8.67 ± 0.57   |
| Acacia catechu         | 8.34 ± 0.77   |
| Zingiber officinale    | 7.78 ± 0.71   |
| Pterocarpus marsupium  | 7.70 ± 0.85   |
| Shorea robusta         | 7.68 ± 0.71   |
| Mangifera indica       | 7.52 ± 1.12   |
| Smallanthus sonchifolius | 7.40 ± 0.83 |
| Myrica esculenta       | 6.84 ± 1.30   |
| Urtica ardens          | 5.89 ± 0.35   |
| Hypericum cordifolium  | 5.89 ± 1.68   |
| Syzygium cumini        | 5.72 ± 0.52   |
| Psidium guajava        | 5.26 ± 1.15   |
| Chrysanthemum indicium | 4.93 ± 0.66   |

TPC of plant extracts was expressed in terms of gallic acid equivalent (mg GAE/gm dry weight of extract) and placed in the order from higher to lower using a calibration curve of gallic acid (y = 0.0025x + 0.0413, R² = 0.981). TPC of plant extracts ranged from 55.21 ± 11.09 to 9.08 ± 1.0 mg GAE/gm. Extract of A. catechu exhibited the highest TPC, followed by U. ardens, M. indica, P. guajava, and S. robusta respectively (Table 4).

Similarly, TFC of plant extracts was expressed in terms of quercetin equivalent (mg QE/gm) and placed in the order from higher to lower using a calibration curve of quercetin (y = 0.0202x - 0.972, R² = 0.972). The extract of E. adenophorum showed the highest TFC (10.23 ± 1.07 mg QE/gm), followed by M. australis and E. prostrata respectively (Table 5).

Antioxidant activity
Free radical scavenging activity was used to assess the antioxidant activity of plant extracts, and the resulting degree of decolorization is stoichiometric in terms of the number of electrons captured from plant extracts.

The results of antioxidant abilities of plant extracts were compared with standard quercetin (IC₅₀ 2.28 µg/mL). Among them, methanolic extract of A. catechu, M. esculenta, S. cumini, and M. indica showed promising antioxidant properties with IC₅₀ ranging 1.3-1.80 µg/mL (Table 6).

Evaluation of antibacterial activity
Plant extracts were examined for antibacterial activity against eight MDR bacteria and four ATCC bacterial species adopting the agar well diffusion technique. The extracts of M. australis, S. robusta, and M. indica showed the largest ZOI i.e. 21 mm at 50 mg/mL towards E. coli ATCC 25922 in agar plates. Meanwhile, only E. prostrata extract showed 7 mm of the ZOI against K. pneumoniae ATCC 700603. The M. australis extract showed 22 mm of the ZOI against S. aureus ATCC 25923, which was the highest among the ZOI shown by plant extract. Similarly, M. australis extract showed the highest ZOI against three MDR bacterial strains, K. pneumoniae, MRSA, and P. aeruginosa with 25 mm, 19 mm, and 20 mm, respectively (Table 8).
Table 6: IC\textsubscript{50} values of plant extracts for antioxidant assay.

| Medicinal plants             | IC\textsubscript{50} (µg/mL) |
|-----------------------------|-----------------------------|
| Smallanthus sonchifolius    | 329.0 ± 0.01                |
| Morus australis             | 208.60 ± 0.02               |
| Pterocarpus marsupium       | 38.50 ± 0.04                |
| Shorea robusta              | 2.50 ± 0.01                 |
| Mangifera indica            | 1.80 ± 0.06                 |
| Syzygium cumini             | 1.60 ± 0.04                 |
| Myrica esculenta            | 1.50 ± 0.03                 |
| Acacia catechu              | 1.30 ± 0.05                 |
| Quercetin (Standard)        | 2.28                        |

Note: only significant results were shown and placed in order from higher to lower IC\textsubscript{50} value.

The extract of *S. robusta* and *M. indica* showed 17 mm of the Zol against MDR *A. baumannii*. Figure 1, presents Zol of plant extracts against ATCC strains *E. coli* and *S. aureus* while Figure 2, presents Zol of plant extracts against the MDR *K. pneumoniae* and *Xanthomonas* species.

Although some plant extracts exhibited potent antimicrobial activity towards some bacterial species, a higher number of plant extracts had a minimum antibacterial effect. The MIC of plant extracts against ATCC strains was between 0.012 mg/mL to 25 mg/mL (Table 9). Extracts of *M. australis* and *H. cordifolium* showed a broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria such as *K. pneumoniae*, *E. coli*, and *S. aureus*. The most potent antibacterial activity (MIC = 0.012 mg/mL) was shown by extracts of *M. australis*, *H. cordifolium*, and *P. guajava*, and the least antibacterial activity (MIC = 25 mg/mL) was observed in extracts of *E. prostrata* and *S. cumini* against ATCC strain of *S. aureus*. Regarding MDR strains, the most potent antibacterial activity (MIC = 0.012 mg/mL) was shown by the extracts of *M. australis* and *H. cordifolium* against *K. pneumoniae* (386), followed by *M. australis* against *Xanthomonas* species (4331) and *P. aeruginosa* (484) (Table 10).
Table 7: Antibacterial activity of plant extracts against ATCC bacterial strains.

| Medicinal plants          | E. coli ATCC 25922 | K. pneumoniae ATCC700603 | S. Typhimurium ATCC 14028 | S. aureus ATCC 25923 |
|---------------------------|--------------------|--------------------------|---------------------------|----------------------|
| Eupoterium adenophorum    | 12                 | -                        | -                         | 11                   |
| Morus australis           | 21                 | -                        | -                         | 22                   |
| Eclipta prostrata         | 9.0                | 7.0                      | -                         | 11                   |
| Acacia catechu            | 18                 | -                        | -                         | 15                   |
| Zingiber officinalae      | -                  | -                        | -                         | -                    |
| Pterocarpus marsupium     | 12                 | -                        | -                         | 14                   |
| Shorea robusta            | 21                 | -                        | -                         | 17                   |
| Mangifera indica          | 21                 | -                        | -                         | 14                   |
| Smallanthus sonchifolius  | -                  | -                        | -                         | -                    |
| Myrica esculenta          | 16                 | -                        | -                         | 19                   |
| Urtica ardens             | -                  | -                        | -                         | -                    |
| Hypericum cordifolium     | 10                 | -                        | -                         | 18                   |
| Syzygium cumini           | 17                 | -                        | -                         | 13                   |
| Psidium guajava           | 16                 | -                        | -                         | 15                   |
| Chrysanthemum indicium    | 8.0                | -                        | -                         | 12                   |
| Neomycin                  | 22                 | 10                       | 15                        | 20                   |
| 50% DMSO                  | -                  | -                        | -                         | -                    |

Diameter of zone of inhibition in mm, well diameter = 6 mm, (-) = No antibacterial activity

Table 8: Antibacterial activity of plant extracts against MDR bacterial strains.

| Medicinal plants          | 2A | 386 | 338 | 628 | 377 | 767 | 4331 | 484 |
|---------------------------|----|-----|-----|-----|-----|-----|------|-----|
| Eupoterium adenophorum    | -  | -   | -   | 13  | -   | 15  | 11   | -   |
| Morus australis           | -  | 25  | 19  | 14  | -   | 15  | -    | 20  |
| Eclipta prostrata         | -  | 10  | -   | 16  | -   | 10  | 9.0  | -   |
| Acacia catechu            | -  | 14  | -   | 12  | -   | 14  | -    | -   |
| Zingiber officinalae      | -  | -   | -   | -   | -   | -   | -    | -   |
| Pterocarpus marsupium     | -  | -   | 13  | 12  | -   | 17  | 11   | -   |
| Shorea robusta            | -  | -   | -   | 17  | -   | -   | -    | -   |
| Mangifera indica          | -  | -   | 17  | -   | 12  | -   | -    | -   |
| Smallanthus sonchifolius  | -  | -   | -   | 9.0 | -   | -   | -    | -   |
| Myrica esculenta          | -  | -   | -   | 13  | -   | 15  | 16   | -   |
| Urtica ardens             | -  | -   | -   | -   | -   | 9   | -    | -   |
| Hypericum cordifolium     | -  | 20  | 12  | 16  | -   | -   | -    | 20  |
| Syzygium cumini           | -  | 16  | 16  | 14  | -   | 17  | -    | -   |
| Psidium guajava           | -  | -   | 12  | 14  | -   | 17  | -    | -   |
| Chrysanthemum indicium    | -  | -   | 15  | 15  | -   | 8.0 | -    | -   |
| Neomycin                  | 15 | 23  | 15  | -   | -   | 11  | 10   | -   |
| 50% DMSO                  | -  | -   | -   | -   | -   | -   | -    | -   |
| Amikacin                  | -  | -   | 23  | 20  | -   | -   | 15   | 23  |
| Nitrofurantoin            | 22 | 18  | 16  | 17  | 16  | -   | 15   | -   |

( ) No antibacterial activity, 2A = E. coli, 338 = methicillin-resistant S. aureus (MRSA), 386 = K. pneumoniae, 628 = A. baumannii, 377 = C. freundii, 767 = Xanthomonas species, 4331 = M. morganii, 484 = P. aeruginosa

Discussion
In developing health care, the search for new medicines with better or enhanced therapeutic actions derived from medicinal plants with ethnobotanical significance has become increasingly valuable [57,58]. Extraction is the most important step in obtaining the plant's bioactive compounds, and the yield is determined by the solvent and extraction method used [59]. In this study, methanol was used as a solvent with a
percentage yield of *H. cordifolium* being the highest (28.46 %) followed by *A. catechu* (23 %) (Table 3). The methanolic extract of *A. catechu* showed the highest TPC, while the extract of *E. adenophorum* showed the highest

| Medicinal plants                        | E. coli ATCC 25922 | K. pneumoniae ATCC 700603 | S. Typhimurium ATCC 14028 | S. aureus ATCC 25923 |
|-----------------------------------------|--------------------|---------------------------|---------------------------|----------------------|
| Eupoterium adenophorum                  |                    |                           |                           |                      |
| Morus australis                         | 3.125              | 6.25                      | -                         | 0.012                |
| Eclipta prostrata                       | 6.25               | -                         | -                         | 25.0                 |
| Acacia catechu                          | 0.39               | -                         | -                         | 6.25                 |
| Zingiber officinale                     |                    |                           |                           |                      |
| Pterocarpus marsupium                   | 12.5               | -                         | -                         | 1.56                 |
| Shorea robusta                          | 3.125              | -                         | -                         | 12.5                 |
| Mangifera indica                        | 0.39               | -                         | -                         | 12.5                 |
| Smallanthus sonchifolius                |                    |                           |                           |                      |
| Myrica esculenta                        | 0.097              | -                         | -                         | 1.56                 |
| Urtica ardens                           |                    |                           |                           |                      |
| Hypericum cordifolium                   | 6.25               | 6.25                      | -                         | 0.012                |
| Syzygium cumini                         | 0.39               | -                         | -                         | 25.0                 |
| Psidium guajava                         | 0.39               | -                         | -                         | 0.012                |
| Chrysanthemum indicium                  | 6.25               | -                         | -                         |                      |
| Neomycin                                | 0.097              | -                         | -                         | 1.56                 |
| 50% DMSO                                |                    |                           |                           |                      |

Diameter of zone of inhibition in mm, well diameter = 6 mm, (-) = No antibacterial activity reported. Neomycin serves as positive control while 50% DMSO serves as a negative control for the test. The concentration of plant extracts expressed in mg/ml.

| Medicinal plants                        | 386 | 338 | 628 | 767 | 4331 | 484 |
|-----------------------------------------|-----|-----|-----|-----|------|-----|
| Eupoterium adenophorum                  | 1.56| -   | -   | -   | -    | -   |
| Morus australis                         | 0.012| 0.19| 3.12| 0.05| -    | 0.05|
| Eclipta prostrata                       | 1.56| -   | 6.25| 3.12| 12.5 | -   |
| Acacia catechu                          | 0.78| -   | 6.25| 1.56| -    | -   |
| Zingiber officinale                     | -   | -   | -   | -   | -    | -   |
| Pterocarpus marsupium                   | 0.39| 1.56| 3.12| 0.39| 12.5 | -   |
| Shorea robusta                          | -   | -   | 6.25| -   | -    | -   |
| Mangifera indica                        | -   | -   | 3.12| 0.78| -    | -   |
| Smallanthus sonchifolius                | -   | -   | 6.25| -   | -    | -   |
| Myrica esculenta                        | 0.39| 12.5| 3.12| 1.56| 6.25 | -   |
| Urtica ardens                           | -   | -   | -   | -   | 12.5 | -   |
| Hypericum cordifolium                   | 0.012| 0.19| 6.25| -   | -    | 0.78|
| Syzygium cumini                         | 0.19| -   | 6.25| 0.78| -    | -   |
| Psidium guajava                         | -   | 3.12| 3.12| 1.56| -    | -   |
| Chrysanthemum indicium                  | -   | 1.56| 6.25| 1.56| -    | -   |
| 50% DMSO                                | -   | -   | -   | -   | -    | -   |
| Neomycin                                | 0.78| 6.25| -   | -   | 12.5 | 0.012|
| Amikacin                                | -   | 3.12| 3.12| -   | 6.25 | 0.78|
| Nitrofurantoin                          | 3.12| -   | -   | 3.12| -    | 0.78|

(·) No antibacterial activity, 2A = E. coli; 338 = methicillin-resistant S. aureus (MRSA), 386 = K. pneumoniae, 628 = A. baumannii, 377 = C. freundii, 767 = Xanthomonas species, 4331 = M. morganii, 484 = P. aeruginosa. Neomycin, Amikacin and Nitrofurantoin were used as positive control and 50% DMSO as negative control for test. The concentration of plant extracts expressed in mg/ml.
TFC values of 55.21 ± 11.09 mg GAE/gm and 10.23 ± 1.07 mg QE/gm respectively (Table 4 and Table 5). A. catechu had the highest free radical scavenging activity in the DPPH assay, followed by M. esculenta, S. cumini, and S. robusta. Flavonoid and phenolic compounds from plants have been shown to have free radical scavenging activity and antioxidant properties, according to previous research [60]. The methanolic extract of A. catechu shows the IC₅₀ of about 84.9 ± 1.9 µg/mL while 1.30 ± 0.05 µg/mL in our study [19]. The difference might be due to environmental variation, temperature, harvesting time, and temperature. These antioxidant mechanisms defend humans from infections and degenerative diseases by inhibiting and scavenging free radicals [61].

The present study showed selected plant extracts possessed antibacterial activity; E. prostrata showed potential antibacterial activity against the ATCC strain of E. coli, S. aureus, and K. pneumoniae with ZoI ranging from 7 mm to 11 mm. Meanwhile, against MDR strains, the extract of E. prostrata showed ZoI against Acinetobacter spp. (628), K. pneumoniae (386), Morganella morganii (4331), and Xanthomonas spp. (767). Previous studies also support the antibacterial and antifungal activity of E. prostrata (Chung et al., 2017; Khanna & Kannabiran, 2008). Cherdtrakulki at al. (2015) reported that bioactive compounds isolated from the aerial parts of E. prostrata such as triterpenoids, 3-acetylaureitolic acid, stigmasterol, a mixture of triterpenoids, fatty esters, and aromatic components, had effective antimicrobial activity against Corynebacterium diphtheria NCTC 10356, Morexella catarrhalis, Streptococcus pyogenes and Saccharomyces cerevisiae ATCC 2601. Another study suggests the presence of alkaloids, cardiglycosides, phytosterol, beta-amyrin, polyacetylene, caffeic acid, stigmasterol, daucosterol on E. prostrata extracts and are found to be effective against K. pneumoniae, S. dysenteriae, E. coli, S. Typhi, B. subtilis, P. aeruginosa, and S. aureus [26]. Recently, eliprostins A, B, and C isolated from this plant showed MIC of 25.0, 6.25 and 25.0 µM, respectively towards the growth of S. aureus [64].

M. australis extract showed a wide range of antibacterial activity against the MDR strains of Acinetobacter spp. (628), methicillin-resistant S. aureus (MRSA) (338), K. pneumoniae (386), P. aeruginosa (484), and Xanthomonas spp. (767) with MIC value of 3.12 mg/mL, 0.19 mg/mL, 0.012 mg/mL, 0.05 mg/mL and 0.05 mg/mL respectively. A similar kind of result was observed by Wei et al. (2016) against a wide range of pathogens such as S. aureus, Fusarium roseum, S. faecalis, B. cereus, E. coli, K. pneumoniae, P. aeruginosa, Salmonella enterica serovar typhi, C. freundii, Candida albicans, Microsporum audouinii, B. subtilis, Micrococcus flavus, and Salmonella abortus due to presence of phytoconstituents such as that mulberrofuran, moracins, oxyresveratrol, morusin, and kuwanon C isolated from methanolic extract of Morus plant's root bark. Other plant extracts such as P. marsupium, M. esculenta, H. cordifolium also exhibited antibacterial activity against MDR strains with varying MIC values (Table 9 and Table 10).

The plant extracts might have a wide variety of phytochemicals that have different mechanisms of action for their antimicrobial activity [66]. By inhibiting enzymes and highly oxidizing compounds, phenol or hydroxylated phenol inhibits bacterial development, likely through reaction with sulphydryl groups or nonspecific interactions with proteins [67]. Antimicrobial effects are possibly due to flavonoids’ ability to bind to extracellular and soluble proteins, as well as bacterial cell walls, inactivate enzymes, and disrupt microbial membranes [68]. Tannins function as antimicrobials by binding to adhesins, inhibiting enzymes, depriving bacteria of their food, forming a complex with the cell wall, disrupting membranes, and complexing metal ions [69]. Terpenoids and essential oils show antimicrobial activity by membrane disruption by the lipophilic compounds. Alkaloid acts as an antimicrobial agent by intercalating into the cell wall and DNA of parasites [10]. These results indicate that Nepalese medicinal plants contain different phytochemicals that need to be explored further to acquire a future drug candidate against MDR pathogens.

Conclusion

Medicinal plants have long been used as traditional healers for a range of infections, and they are also useful in the formulation of drugs to treat a variety of conditions. The leaves extract of E. andenophorum showed the highest TFC (10.23 ± 1.07 mg QE/gm) while bark extract of A. catechu showed a high TPC (55.21 ± 11.09 mg GAE/gm). Morus australis showed a broad-spectrum antibacterial activity that might be a potential source of the future drug to treat MDR-associated infections. Similarly, other plant extracts such as E. prostrata, M. esculenta, P. marsupium, and H. cordifolium also showed potential antibacterial activity against clinical isolates of MDR bacteria. Future studies are anticipated to examine the possibility of these plants in
ethnomedicine and drug discovery to treat infections caused by drug-resistant pathogens.

Availability of data and materials
Plant specimen herbaria are kept in the National College, Kathmandu, and can be retrieved as needed. Data supporting this manuscript are accessible upon appropriate request to the corresponding author.

Conflict of interests
We announce that none of the writers have a conflict of interest in reporting these results.

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List of abbreviations
American type culture collection (ATCC); Minimum inhibitory concentrations (MIC); Multidrug-resistant (MDR); Optical density (OD); The inhibitory concentration of drug/extract that gives half-maximal response (IC₅₀); Total phenol contents (TPC); Total flavonoid contents (TFC); Zone of inhibition (ZoI); 2,2-diphenyl-1-picrylhydrazyl (DPPH)

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