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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 *Eupoterium 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 IC_{50} value of 1.3 µg/mL, followed by extracts of *Myrica 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

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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
Smallanthus 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 indicium	NCDB205	Godawari	Leaves
Myrica esculenta	NCDB208	Kafal	Leaves
Urtica ardens	NCDB213	Sisnoo	Buds
Pterocarpus marsupium	NCDB204	Bijayasal	Barks
Eupoterium adenophoium	NCDB202	Banmara	Leaves
Zingiber officinale	NCDB200	Aaduwa	Leaves
Acacia 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, astringent, 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 extract) 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 x10^8 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 (50µL) 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.

Table 2: Medicinal plants selected understudy 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-yne-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, 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	Used as a functional food, antioxidant, antimicrobial, prebiotic, growth promoter [30]. Leaves extract contains the compounds fluctuanin 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. austrralis* 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, caries. The hypoglycemic components in *Psidium guajava* might be due to oleanolic acid, arjunolic acid, ursolic acid, and glucuronic acid [40].
Chrysanthemum indicium	Asteraceae	Used for hypertension, pneumonia, colitis, stomatitis, fever, neurological problems, headache [41], antipyretic purpose, 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].
Eupoterium adenophoium	Asteraceae	Used for treatment of emetic, diaphoretic, stimulant, tonic, fever, cuts and wounds, analgesic [49]. Used as an anti-inflammatory, blood coagulant, antimicrobial, antiseptic, and analgesic, antipyretic. Isomers of mono-caffeoylquinic acid present in *E. adenophoium* exhibit potent anti-inflammatory, anti-bacterium, and anti-obesity properties [50].
Zingiber officinale	Zingiberaceae	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].
Acacia catechu	Fabaceae	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].
Syzygium cumini	Myrtaceae	Used for diabetes mellitus, constipation, stomachache, HIV, inflammation leucorrhoea, fever, strangury, and dermopathy [54], [55]. Ferulic acid and Catechins possess antioxidant properties [56]. Gallocatechins 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, R^2 = 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, R^2 = 0.972). **T**he 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 (IC_{50} 2.28 µg/mL). Among them, methanolic extract of *A. catechu, M. esculenta, S. cumini,* and *M. indica* showed promising antioxidant properties with IC_{50} 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_{50} values of plant extracts for antioxidant assay.

Medicinal plants	IC_{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_{50} value.

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

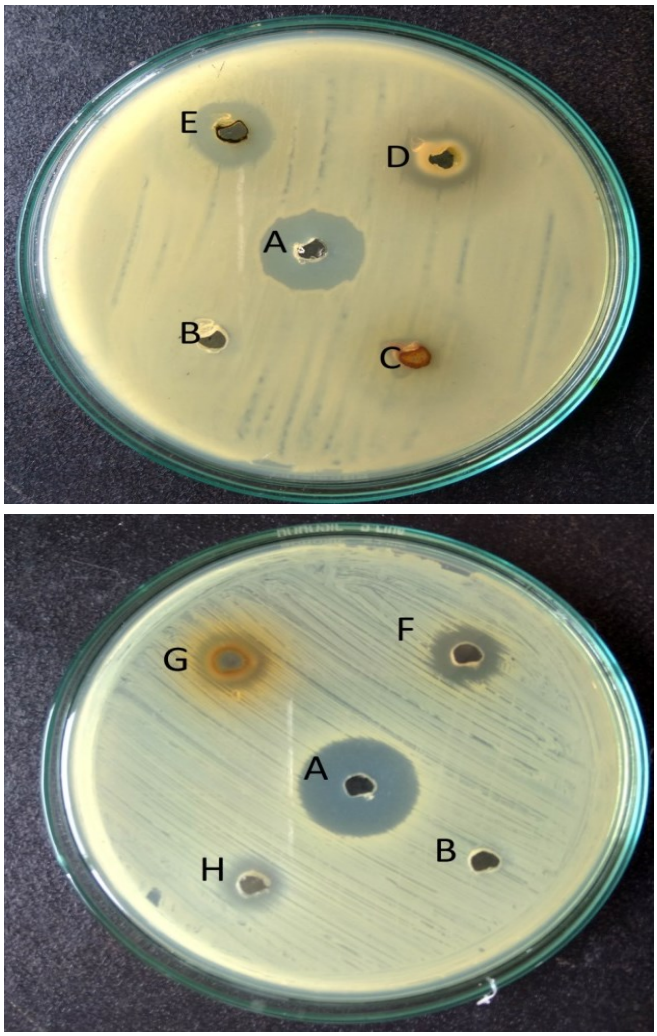

Figure 1. Antibacterial activity of plant extracts against ATCC organism E. coli and S. aureus: A) Neomycin; B) 50% DMSO; C) E. prostrata; D) P. marsupium; E) A. catechu: F) M. indica; G) S. robusta; H) C. indicium.

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**).

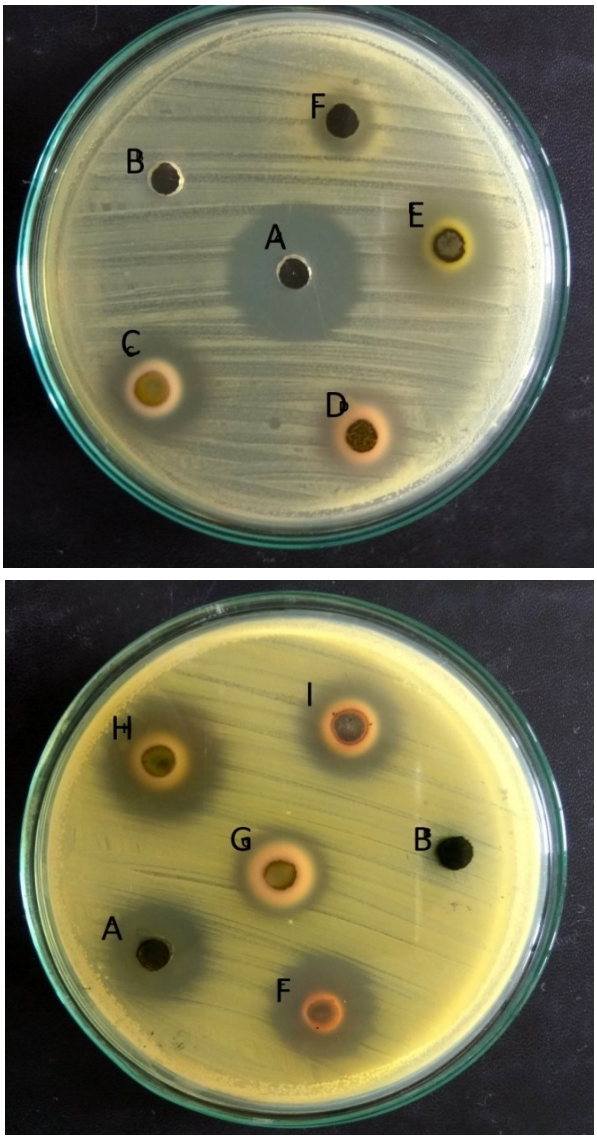

Figure 2. Antibacterial activity of plant extracts against MDR *K.pneumoniae* and *Xanthomonas* species; A) Neomycin; B) 50% DMSO; C) *H. cordifolium;* D) *S. cumini;* E) *M. australis:* F) *A. catechu;* G) *M. indica;* H) *P. marsupium;* I) *M. esculenta.*

Table 7: Antibacterial activity of plant extracts against ATCC bacterial strains.

Medicinal plants	Bacterial strains							
	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 officinale	-	-	-				-	
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	Bacterial strains							
	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 officinale	-	-	-	-	-	-	-	-
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. pnemoniae*, 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

Table 9: MIC of plant extracts against ATCC reference strains.

Medicinal plants	Bacterial strains			
	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	0.39	3.12	0.78	0.39
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.

Table 10: MIC of plant extracts against MDR bacterial strains.

Medicinal plants	Bacterial strains					
	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.

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

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 IC_{50} 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. prostata* (Chung et al., 2017; Khanna & Kannabiran, 2008). Cherdtrakulki at et al. (2015) reported that bioactive compounds isolated from the aerial parts of *E. prostrat*a such as triterpenoids, 3-acetylaleuritolic 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, ecliprostins 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*, *Micrococus flavus*, and *Salmonella abony* 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 sulfhydryl groups or nonspecific interactions with proteins [67]. Antimicrobial effects are possibly due to flavonoid's 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.

Funding statement

Not applicable

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 (IC_{50}); Total phenol contents (TPC); Total flavonoid contents (TFC); Zone of inhibition (ZoI); 2,2-diphenyl-1-picrylhydrazyl (DPPH)

References

1. WHO. (2019). *WHO global report on traditional and complementary medicine 2019*. World Health Organization. https://apps.who.int/iris/handle/10665/312342
2. Kirbağ SE, Zengin F, Kursat M. (2009). Antimicrobial activities of extracts of some plants. *Pakistan J. of Botany*, 41(4), 2067-70.
3. Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268–281. https://doi.org/10/bbqpzj
4. Tavares, L. S., Silva, C. dos S. F. da, Souza, V. C., Silva, V. L. da, Diniz, C. G., & Santos, M. D. O. (2013). Strategies and molecular tools to fight antimicrobial resistance: Resistome, transcriptome, and antimicrobial peptides. *Frontiers in Microbiology*, *4*, 412. https://doi.org/10/ghf5qc
5. Sommer, M. O., & Dantas, G. (2011). Antibiotics and the resistant microbiome. *Current Opinion in Microbiology*, *14*(5), 556–563. https://doi.org/10/b6ttxr
6. Lima, R., Del Fiol, F. S., & Balcão, V. M. (2019). Prospects for the Use of New Technologies to Combat Multidrug-Resistant Bacteria. *Frontiers in Pharmacology*, *10*. https://doi.org/10.3389/fphar.2019.00692
7. Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M. K. F., & Baloch, Z. (2018). Antibiotic resistance: A rundown of a global crisis. *Infection and Drug Resistance*, *11*, 1645–1658. https://doi.org/10.2147/IDR.S173867
8. Tagliabue, A., & Rappuoli, R. (2018). Changing Priorities in Vaccinology: Antibiotic Resistance Moving to the Top. *Frontiers in Immunology*, *9*, 1068. https://doi.org/10.3389/fimmu.2018.01068
9. Mulani, M. S., Kamble, E. E., Kumkar, S. N., Tawre, M. S., & Pardesi, K. R. (2019). Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Frontiers in Microbiology*, *10*, 539. https://doi.org/10/ghfddk
10. Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Frontiers in Microbiology*, *10*, 911. https://doi.org/10.3389/fmicb.2019.00911
11. Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, *31*(4), 247–256. https://doi.org/10.1590/S1517-83822000000400003
12. Blanco, M. M., Costa, C. A. R. A., Freire, A. O., Santos, J. G., & Costa, M. (2009). Neurobehavioral effect of essential oil of Cymbopogon citratus in mice. *Phytomedicine*, *16*(2), 265–270. https://doi.org/10.1016/j.phymed.2007.04.007
13. Bekoe, E. O., Kitcher, C., Gyima, N. A. M., Schwinger, G., & Frempong, M. (2018). Medicinal Plants Used as Galactagogues. *Pharmacognosy - Medicinal Plants*. https://doi.org/10/ghf3rm
14. Işcan, G., Kirimer, N., Kürkcüoğlu, M., Hüsnü Can Başer, & Demirci, F. (2002). Antimicrobial Screening of *Mentha piperita* Essential Oils. *Journal of Agricultural and Food Chemistry*, *50*(14), 3943–3946. https://doi.org/10/cs7jf8
15. Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, *2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102
16. Bhandari, S., Khadayat, K., Poudel, S., Shrestha, S., Shrestha, R., Devkota, P., Khanal, S., & Marasini, B. P. (2021). Phytochemical analysis of medicinal plants of Nepal and their antibacterial and antibiofilm activities against uropathogenic Escherichia coli. *BMC Complementary Medicine and Therapies*, *21*(1), 116. https://doi.org/10/gk45f5
17. Joubert, E., Manley, M., & Botha, M. (2008). Evaluation of spectrophotometric methods for screening of green rooibos (Aspalathus linearis) and green honeybush (Cyclopia genistoides) extracts for high levels of Bio-active compounds. *Phytochemical Analysis*, *19*(2), 169–178. https://doi.org/10/dv3t8n
18. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate

antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
19. Aryal, B., Niraula, P., Khadayat, K., Adhikari, B., Chhetri, D., Sapkota, B., Bhattarai, B., & Parajuli, N. (2021). Antidiabetic, Antimicrobial, and Molecular Profiling of Selected Medicinal Plants. *Evidence-Based Complementary and Alternative Medicine*, *2021*. https://doi.org/10/gjxhzt
20. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005
21. Marasini, B. P., Baral, P., Aryal, P., Ghimire, K. R., Neupane, S., Dahal, N., Singh, A., Ghimire, L., & Shrestha, K. (2015, February 9). *Evaluation of Antibacterial Activity of Some Traditionally Used Medicinal Plants against Human Pathogenic Bacteria* [Research Article]. BioMed Research International; Hindawi. https://doi.org/10.1155/2015/265425
22. Valgas, C., Souza, S. M. de, Smânia, E. F. A., & Smânia Jr., A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, *38*(2), 369–380. https://doi.org/10/ftmw7n
23. Kato, M., Sugiyama, K., Fukushima, T., Miura, Y., Awogi, T., Hikosaka, S., Kawakami, K., Nakajima, M., Nakamura, M., Sui, H., Watanabe, K., & Hakura, A. (2018). Negative and positive control ranges in the bacterial reverse mutation test: JEMS/BMS collaborative study. *Genes and Environment*, *40*(1), 7. https://doi.org/10.1186/s41021-018-0096-1
24. CLSI. (2015). *Performance standards for antimicrobial susceptibility testing: 25th informational supplement (M100-S23)*. Clinical and Laboratory Standards Institute.
25. Mors, W. B., do Nascimento, M. C., Parente, J., da Silva, M. H., Melo, P. A., & Suarez-Kurtz, G. (1989). Neutralization of lethal and myotoxic activities of South American rattlesnake venom by extracts and constituents of the plant Eclipta prostrata (Asteraceae). *Toxicon*, *27*(9), 1003–1009. https://doi.org/10.1016/0041-0101(89)90151-7
26. Priya, K., John, P., Usha, P. T. A., Kariyil, B. J., Uma, R., & Hogale, M. S. (2018). Phytochemical Analysis of Eclipta prostrata L. (L.) Leaves. *International Journal of Current Microbiology and Applied Sciences*, *7*(08), 1069–1075. https://doi.org/10.20546/ijcmas.2018.708.121
27. Chung, I.-M., Rajakumar, G., Lee, J.-H., Kim, S.-H., & Thiruvengadam, M. (2017). Ethnopharmacological uses, phytochemistry, biological activities, and biotechnological applications of Eclipta prostrata. *Applied Microbiology and Biotechnology*, *101*(13), 5247–5257. https://doi.org/10.1007/s00253-017-8363-9
28. Kumar, D., Wani, T., Kumar, D., Prasad, R., Verma, P., Sardar, K., & Tandan, S. (2012). Analgesic activity of the ethanolic extract of Shorea robusta resin in experimental animals. *Indian Journal of Pharmacology*, *44*(4), 493. https://doi.org/10.4103/0253-7613.99322
29. Liu, J. (1995). Pharmacology of oleanolic acid and ursolic acid. *Journal of Ethnopharmacology*, *49*(2), 57–68. https://doi.org/10.1016/0378-8741(95)90032-2
30. Hong, S. S., Lee, S. A., Han, X. H., Lee, M. H., Hwang, J. S., Park, J. S., Oh, K.-W., Han, K., Lee, M. K., Lee, H., Kim, W., Lee, D., & Hwang, B. Y. (2008). Melampolides from the Leaves of Smallanthus sonchifolius and Their Inhibitory Activity of LPS-Induced Nitric Oxide Production. *Chemical and Pharmaceutical Bulletin*, *56*(2), 199–202. https://doi.org/10.1248/cpb.56.199
31. Genta, S. B., Cabrera, W. M., Mercado, M. I., Grau, A., Catalán, C. A., & Sánchez, S. S. (2010). Hypoglycemic activity of leaf organic extracts from Smallanthus sonchifolius: Constituents of the most active fractions. *Chemico-Biological Interactions*, *185*(2), 143–152. https://doi.org/10.1016/j.cbi.2010.03.004
32. Paudel, M. (1970). Non-timber forest products from community forestry practices, problems and prospects for livelihood strategy in Jumla. *Banko Janakari*, *17*(2), 45–54. https://doi.org/10.3126/banko.v17i2.2155
33. Taylor, R. S. L., Manandhar, N. P., Hudson, J. B., & Towers, G. H. N. (1996). Antiviral activities of Nepalese medicinal plants. *Journal of Ethnopharmacology*, *52*(3), 157–163. https://doi.org/10.1016/0378-8741(96)01409-2
34. Núñez-Sellés, A. J. (2005). Antioxidant therapy: Myth or reality? *Journal of the Brazilian Chemical Society*, *16*(4), 699–710. https://doi.org/10.1590/S0103-50532005000500004
35. Shah, K., Patel, M., Patel, R., & Parmar, P. (2010). Mangifera Indica (Mango). *Pharmacognosy Reviews*, *4*(7), 42. https://doi.org/10.4103/0973-7847.65325
36. Jhaumeer Laulloo, S., Bhowon, M. G., Soyfoo, S., & Chua, L. S. (2018). Nutritional and Biological Evaluation of Leaves of *Mangifera indica* from Mauritius. *Journal of Chemistry*, *2018*, 1–9. https://doi.org/10.1155/2018/6869294
37. Katsube, T., Imawaka, N., Kawano, Y., Yamazaki, Y., Shiwaku, K., & Yamane, Y. (2006). Antioxidant flavonol glycosides in mulberry (Morus alba L.) leaves isolated based on LDL antioxidant activity. *Food Chemistry*, *97*(1), 25–31. https://doi.org/10.1016/j.foodchem.2005.03.019
38. Hikino, H., Takahashi, M., Oshima, Y., & Konno, C. (1988). Isolation and Hypoglycemic Activity of Oryzabrans A, B, C and D, Glycans of *Oryza sativa* Bran. *Planta Medica*, *54*(01), 1–3. https://doi.org/10.1055/s-2006-962316
39. Heinrich, M., Ankli, A., Frei, B., Weimann, C., & Sticher, O. (1998). Medicinal plants in Mexico: Healers' consensus and cultural importance. *Social Science & Medicine*, *47*(11), 1859–1871. https://doi.org/10.1016/S0277-9536(98)00181-6
40. Díaz-de-Cerio, E., Verardo, V., Gómez-Caravaca, A., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2016). Exploratory Characterization of Phenolic

Compounds with Demonstrated Anti-Diabetic Activity in Guava Leaves at Different Oxidation States. *International Journal of Molecular Sciences*, *17*(5), 699. https://doi.org/10.3390/ijms17050699

41. Shunying, Z., Yang, Y., Huaidong, Y., Yue, Y., & Guolin, Z. (2005). Chemical composition and antimicrobial activity of the essential oils of Chrysanthemum indicum. *Journal of Ethnopharmacology*, *96*(1–2), 151–158. https://doi.org/10.1016/j.jep.2004.08.031
42. Lee, D. Y., Choi, G., Yoon, T., Cheon, M. S., Choo, B. K., & Kim, H. K. (2009). Anti-inflammatory activity of Chrysanthemum indicum extract in acute and chronic cutaneous inflammation. *Journal of Ethnopharmacology*, *123*(1), 149–154. https://doi.org/10.1016/j.jep.2009.02.009
43. Indra, D. B., & Uppe, D. (2004). Factors controlling micropropagation of Myrica esculenta buch. Ham. ex D. Don: A high value wild edible of Kumaun Himalaya. *African Journal of Biotechnology*, *3*(10), 534–540. https://doi.org/10.5897/AJB2004.000-2097
44. Gyawali, R., Adhikari, S., Gautam, S., Guragain, P., Pokharel, S., Pradhan, N., Sijapati, S., & Shrestha, T. M. (2015). Antimicrobial and cytotoxic properties of selected medicinal plants from Kavrepalanchowk, Nepal. *Banko Janakari*, *25*(1), 15–19. https://doi.org/10.3126/banko.v25i1.13467
45. Taylor, K. (2009). Biological Flora of the British Isles: *Urtica dioica* L. *Journal of Ecology*, *97*(6), 1436–1458. https://doi.org/10.1111/j.1365-2745.2009.01575.x
46. Ibrahim, M., Rehman, K., Razzaq, A., Hussain, I., Farooq, T., Hussain, A., & Akash, M. S. H. (2018). Investigations of Phytochemical Constituents and Their Pharmacological Properties Isolated from the Genus Urtica: Critical Review and Analysis. *Critical Reviews in Eukaryotic Gene Expression*, *28*(1), 25–66. https://doi.org/10.1615/CritRevEukaryotGeneExpr.2018020389
47. Mankani, K. L., Krishna, V., Manjunatha, B. K., Vidya, S. M., Singh, S. J., Manohara, Y. N., Raheman, A.-U., & Avinash, K. R. (2005). Evaluation of hepatoprotective activity of stem bark of Pterocarpus marsupium Roxb. *Indian Journal of Pharmacology*, *37*(3), 165. https://doi.org/10.4103/0253-7613.16213
48. Jung, M., Park, M., Lee, H., Kang, Y.-H., Kang, E., & Kim, S. (2006). Antidiabetic Agents from Medicinal Plants. *Current Medicinal Chemistry*, *13*(10), 1203–1218. https://doi.org/10.2174/092986706776360860
49. Negi, A., Upadhyay, A., Semwal, A., & Kumar Wahi, A. (2010). Pharmacognostical Studies on the Leaves of Eupatorium adenophorum Spreng. *Pharmacognosy Journal*, *2*(15), 1–7. https://doi.org/10.1016/S0975-3575(10)80071-9
50. Liu, B., Cao, L., Zhang, L., Yuan, X., & Zhao, B. (2016). Preparation, Phytochemical Investigation, and Safety Evaluation of Chlorogenic Acid Products from Eupatorium adenophorum. *Molecules*, *22*(1), 67. https://doi.org/10.3390/molecules22010067
51. Grzanna, R., Lindmark, L., & Frondoza, C. G. (2005). Ginger—An Herbal Medicinal Product with Broad Anti-Inflammatory Actions. *Journal of Medicinal Food*, *8*(2), 125–132. https://doi.org/10.1089/jmf.2005.8.125
52. Singh, B. K., Tripathi, M., Chaudhari, B. P., Pandey, P. K., & Kakkar, P. (2012). Natural Terpenes Prevent Mitochondrial Dysfunction, Oxidative Stress and Release of Apoptotic Proteins during Nimesulide-Hepatotoxicity in Rats. *PLoS ONE*, *7*(4), e34200. https://doi.org/10.1371/journal.pone.0034200
53. Negi, B. S., & Dave, B. P. (2010). In Vitro Antimicrobial Activity of Acacia catechu and Its Phytochemical Analysis. *Indian Journal of Microbiology*, *50*(4), 369–374. https://doi.org/10.1007/s12088-011-0061-1
54. Schoenfelder, T., Warmlin, C. Z., Manfredini, M. S., Pavei, L. L., Réus, J. V., Tristão, T. C., Fernandes, M. S., & Costa-Campos, L. (2010). Hypoglycemic and hypolipidemic effect of leaves from Syzygium cumini (L.) Skeels, Myrtaceae. In diabetic rats. *Revista Brasileira de Farmacognosia*, *20*(2), 222–227. https://doi.org/10.1590/S0102-695X2010000200014
55. Ruan, Z. P., Zhang, L. L., & Lin, Y. M. (2008). Evaluation of the Antioxidant Activity of Syzygium cumini Leaves. *Molecules*, *13*(10), 2545–2556. https://doi.org/10.3390/molecules13102545
56. Chagas, V. T., França, L. M., Malik, S., & Paes, A. M. de A. (2015). Syzygium cumini (L.) skeels: A prominent source of bioactive molecules against cardiometabolic diseases. *Frontiers in Pharmacology*, *6*. https://doi.org/10.3389/fphar.2015.00259
57. Hasan, M. M., Hossain, A., Shamim, A., & Rahman, M. M. (2017). Phytochemical and pharmacological evaluation of ethanolic extract of Lepisanthes rubiginosa L. leaves. *BMC Complementary and Alternative Medicine*, *17*(1), 1–11. https://doi.org/10.1186/s12906-017-2010-y
58. Kunwar, R. M., & Bussmann, R. W. (2008). Ethnobotany in the Nepal Himalaya. *Journal of Ethnobiology and Ethnomedicine*, *4*, 1–8. https://doi.org/10.1186/1746-4269-4-24
59. Turkmen, N., Sari, F., & Velioglu, Y. S. (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chemistry*, *99*(4), 835–841. https://doi.org/10.1016/j.foodchem.2005.08.034
60. Khorasani Esmaeili, A., Mat Taha, R., Mohajer, S., & Banisalam, B. (2015, May 6). *Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from In Vivo and In Vitro Grown Trifolium pratense L. (Red Clover)* [Research Article]. BioMed Research International; Hindawi. https://doi.org/10.1155/2015/643285
61. [61] Reznick, A. Z., Shehadeh, N., Shafir, Y., & Nagler, R. M. (2006). Free radicals related effects and antioxidants in saliva and serum of adolescents with Type 1 diabetes mellitus. *Archives of Oral*

Biology, *51*(8), 640–648. https://doi.org/10.1016/j.archoralbio.2006.02.004
62. Khanna, V. G., & Kannabiran, K. (2008). Antimicrobial activity of saponin fractions of the leaves of Gymnema sylvestre and Eclipta prostrata. *World Journal of Microbiology and Biotechnology*, *24*(11), 2737. https://doi.org/10.1007/s11274-008-9758-7
63. Cherdtrakulkiat, R., Boonpangrak, S., Pingaew, R., Prachayasittikul, S., Ruchirawat, S., & Prachayasittikul, V. (2015). Bioactive triterpenoids, antimicrobial, antioxidant and cytotoxic activities of Eclipta prostrata Linn. -. *Journal of Applied Pharmaceutical Science*, *5*(3), 046–050.
64. Yu, S.-J., Yu, J.-H., He, F., Bao, J., Zhang, J.-S., Wang, Y.-Y., & Zhang, H. (2020). New antibacterial thiophenes from Eclipta prostrata. *Fitoterapia*, *142*, 104471. https://doi.org/10.1016/j.fitote.2020.104471
65. Wei, H., Zhu, J.-J., Liu, X.-Q., Feng, W.-H., Wang, Z.-M., & Yan, L.-H. (2016). Review of bioactive compounds from root barks of Morus plants (Sang-Bai-Pi) and their pharmacological effects. *Cogent Chemistry*, *2*(1). https://doi.org/10.1080/23312009.2016.1212320
66. Adeonipekun, P. A., Adeniyi, T. A., & Aminu, S. O. (2014). Investigating the Phytochemicals and Antimicrobial Activities of Shoot and Root of Pycreus smithianus (Ridl.) C. B. Clarke (Family Cyperaceae). *Journal of Botany*, *2014*, 1–5. https://doi.org/10.1155/2014/761613
67. Bouarab-Chibane, L., Forquet, V., Lantéri, P., Clément, Y., Léonard-Akkari, L., Oulahal, N., Degraeve, P., & Bordes, C. (2019). Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure–Activity Relationship) Models. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00829
68. Takó, M., Kerekes, E. B., Zambrano, C., Kotogán, A., Papp, T., Krisch, J., & Vágvölgyi, C. (2020). Plant Phenolics and Phenolic-Enriched Extracts as Antimicrobial Agents against Food-Contaminating Microorganisms. *Antioxidants*, *9*(2), 165. https://doi.org/10.3390/antiox9020165
69. Vu, T. T., Kim, H., Tran, V. K., Vu, H. D., Hoang, T. X., Han, J. W., Choi, Y. H., Jang, K. S., Choi, G. J., & Kim, J.-C. (2017). Antibacterial activity of tannins isolated from Sapium baccatum extract and use for control of tomato bacterial wilt. *PLOS ONE*, *12*(7), e0181499. https://doi.org/10.1371/journal.pone.0181499